THE PRODUCTION OF ANTIBODIES

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THE PRODUCTION OF ANTIBODIES

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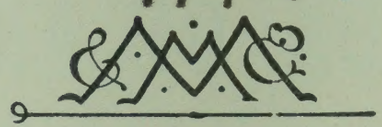

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PREFACE

This is a revised edition of the first monograph from the Walter and Eliza Hall Institute which was published in 1941. In its original form it contained in addition to a review of the literature and a theoretical discussion for which one of us (F. M. Burnet) was wholly responsible, an account of a good deal of experimental work in which Mavis Freeman, A. V. Jackson and Dora Lush collaborated. In preparing the present revision we have thought it advisable to preserve most of this experimental material, which has not been published elsewhere, in its original form. Portion dealing with the production of antibody in lymph nodes has been eliminated since this field has been well covered by other workers.

The general approach to the problem of antibody production that was adopted in 1941, namely, that antibody formation is a biological phenomenon to be interpreted on biological rather than chemical or pseudo-chemical lines, seems to have become increasingly valid. There has been much relevant work and speculation in the meantime in regard to the genetic control of enzymes and antigens and the processes of protein synthesis in the cell. This has provided a more direct logical approach to the problem than was available in 1941 and the form of our discussion is correspondingly altered.

The other big development in the interim has been the change of emphasis from reticulo-endothelial cells to lymphocytes and plasma cells as the probable producers of antibody. This has necessitated a complete rewriting of the relevant section and we hope that our attempt to assess the relation and significance of these three types of cell as they occur in spleen and lymph nodes may point the way to an acceptable synthesis of what at present appear to be conflicting views.

The absence of antigenicity of the body's own con-

stituents and the failure of mammalian or avian embryos to produce antibody are two aspects that have not previously been seriously considered in relation to immunological theory. The introduction of the "self-marker" hypothesis to bring these aspects into the picture is the chief novelty of our presentation. It remains to be seen whether this concept is of value in stimulating further research.

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CHAPTER I

INTRODUCTION

IMMUNOLOGY arose from the study of the immunity known from time immemorial to follow certain infectious diseases. For a long period interest in the phenomena was restricted to their bearing on the practical problems of medical and veterinary practice. Jenner's discovery of vaccination, itself a direct development of the practice of variolation, provided the basic theme for immunological work in the period of Pasteur and Koch. The discovery of antitoxin by Behring followed by that of other types of circulating antibody opened a new phase in which the serum reactions were developed on the one hand for diagnostic and therapeutic use in medicine and on the other as a basis for theories on the nature of recovery from infection. The development of knowledge about phagocytosis led to a prolonged controversy between proponents of the humoral and cellular theories of immunity but with insignificant exceptions interest remained tied to the directly medical aspects of the problem.

The next phase, especially associated with the name of Landsteiner, was the opening up of a chemical approach to the nature of the test tube reactions between antigen and antibody. This has been greatly accelerated by the development of methods for studying the physical chemistry of proteins on the one hand, and by the general advance in the chemistry of complex biological molecules on the other. Antibodies were purified and characterized as globulin molecules, and the specificity of reaction was established as due to the chemical structure of the antigen by the use of the artificial antigens of Landsteiner and others. This approach has been and continues to be highly fruitful in theoretical and practical results but it has still left immunology as a science almost wholly unrelated to the general pattern of biological knowledge.

The present dominant theoretical conception of anti-

body production, due to Breinl and Haurowitz (1930), Mudd (1932), and Pauling (1940), is in itself an indication of how slender is the link between immunology and the biological sciences. It is concerned only with an attempt to picture a mechanism by which a globulin molecule can be endowed with a surface configuration complementary to that of the antigen concerned. The view adopted that the antigenic molecules or at least their determinant groups persist in the antibody-forming cells as long as any antibody is being produced seems to us to be quite out of keeping with the anatomical facts. Unless the antigen or its determinants can be so built into the cellular mechanism that it is capable of multiplication through successive cell generation there is no conceivable place in the body where it can be stored. Nor does this theory provide any framework for an interpretation of the difference between primary and secondary types of response or the changing character of antibody in the course of repeated immunization.

In the present monograph as in its predecessor we are not concerned with the chemical basis of antibody specificity nor with the physical chemistry of the antigen-antibody reaction except insofar as they bear on the problem of antibody formation. These fields have been well reviewed by Landsteiner (1946), Marrack (1938), Treffers (1947) and Pauling *et al.* (1943). A brief account of modern work on the chemical character of typical serum antibodies is included in this introductory chapter but the rest of the work is wholly concerned with the evidence bearing on the processes of antibody formation and an attempt to interpret these findings in biological rather than chemical terms.

THE GENERAL CHARACTER OF ANTIBODIES

At the present time it is accepted that the specific activity of immune serum is a function of globulin molecules which differ from other molecules of the same general type in being specifically adapted to unite with the corresponding antigen. Modern developments of immunology have left the terms "antibody" and "antibodies" rather

ambiguous, particularly when the not infrequent question arises as to whether a given serum contains one antibody or a multiplicity of antibodies. In this discussion we shall use "antibody" in the current loose sense as the factor in an immune serum responsible for its specific behaviour, "antibody molecules" for molecules of specifically modified globulin, and "antibody type" as a collective name for the antibody molecules specifically adapted to unite with a single antigen.

As Grabar (1946) has emphasized in a recent review on the globulins, normal mammalian serum contains a very complicated mixture of proteins, many of which form loose complexes with lipids, polysaccharides or other proteins. The serum proteins have been divided into two principal components on the basis of solubility in 50 per cent ammonium sulphate. The soluble portion is the serum albumin which is not concerned with antibody function and need not be further considered.

The insoluble fraction, the globulin, is by no means homogeneous and a large proportion of recent work on the physical properties of serum has been concerned with the characterization of various globulin components. The methods chiefly used, electrophoretic analysis, determination of sedimentation constants in the ultracentrifuge and fractional precipitation by various methods, will each allow the differentiation of certain fractions. It is, however, not usually possible to equate completely the fractions obtained by one method with those obtained by another. There are also considerable differences in the pattern of fractionation obtained from one species of animal to another. The impression received in working through the literature of the subject is that the "globulin" includes a very heterogeneous collection of protein molecules including many with close relation to lipid and polysaccharide serum components. Physical methods separate these molecules into fractions depending primarily on the particular physical character concerned but also greatly influenced by factors such as ionic concentrations and interaction with other components of the serum.

Studies with the ultracentrifuge have shown that in di-

luted normal serum there are two major components, one of which sediments at a rate corresponding to a molecular weight of about 70,000 (serum albumin) while the other component with an indicated molecular weight of about 160,000 corresponds to the globulin fraction. When undiluted serum is used as in McFarlane's (1935) experiments the proportion of "globulin" is lower, perhaps suggesting that in dilution a certain degree of aggregation occurs. In normal serum there are small amounts of heavier molecules the most prominent component having a molecular weight of about 900,000.

The most generally used classification is that based upon the behaviour of serum in an electrical field. It is found by such electrophoretic analysis that the most rapidly moving component corresponds to the albumin and that the globulin fraction is represented by three principal components migrating at different rates α, β and γ globulin (Tiselius 1937). More delicate analysis (Wiedemann 1945, 1946) has shown that in human serum there are two α globulins, three β globulins and two γ globulins, and similar subfractions have been found in horse serum (Svensson 1941).

Electrophoretic analysis of immune sera has shown that antibodies in general migrate with the γ globulin (van der Scheer, Bohnel, Clarke, and Wyckoff 1942), though in certain cases they migrate with β globulin, e.g. a proportion of diphtheria antitoxin in horse serum (Kekwick and Record 1941) or with a speed intermediate between β and γ globulin, as with tetanus antitoxin produced in the horse (van der Scheer, Wyckoff and Clarke 1941). In γ globulin prepared from pooled human sera Enders (1944) recognized sixteen different antibodies (antitoxic, antibacterial and anti-viral), and there are probably many more that were not sought. Even in human sera, however, certain antibodies, e.g. the Wassermann antibody (Davis, Moore, Kabat, and Harris 1945), typhoid O antibody and the normal isoagglutinins (Deutsch, Alberty, Gosting, and Williams 1947) migrate with a speed between the β and γ globulins and appear to consist largely of high molecular weight components.

The type-specific antibody produced in horses immunized with pneumococci is a protein with sharply defined differences from the predominant normal globulin of the horse, and has been very intensively studied. It is readily precipitated from serum by simple dilution with water, and can be obtained in highly purified form. In the ultracentrifuge it sediments rapidly, and in recently immunized animals the molecules are homodisperse with a molecular weight of 990,000. Purified solutions show an easily demonstrable birefringence of flow which, with other confirmatory physical evidence, indicates that the molecules are not spherical but probably rather slender cylinders (Kabat 1939). In normal horse serum there is a very small component of the same molecular weight and physico-chemical properties (Heidelberger and Pedersen 1937). In its electrophoretic behaviour this antibody type usually corresponds with the γ globulin (van der Scheer, Lagsdin and Wyckoff 1941), but occasionally it migrates with a new component (β_2 or T) (Tiselius and Kabat 1939) which is characteristically produced in horses immunized by subcutaneous or intramuscular injection of toxins or toxoids (van der Scheer, Wyckoff and Clarke 1941). Antipneumococcal antibody produced by other ungulates (cow and goat) resembles that of the horse in having a molecular weight just under 1,000,000.

The same antibody type produced in man, monkey or rabbit is composed of γ globulin molecules of normal size (150-160,000) (Kabat 1939), (van der Scheer, Bohnel, Clarke and Wyckoff 1942). The precipitin produced by immunization of rabbits with crystalline ovalbumin is also physically a normal globulin and can be shown to migrate electrophoretically with the γ component, and to be present only in this component (Tiselius and Kabat 1939).

We may conclude from the available evidence that antibody molecules in general correspond physically to one or other type of globulin molecule found in the normal serum of the animal species subjected to the antigenic stimulus. Occasionally the antibody molecules differ from any component normally recognizable in significant

amounts. The physical nature of the globulin molecules endowed with antibody specificity varies with the species immunized, the route of injection, and the type of antigen. These facts have the important implication that different components of the serum globulin are produced by different cells, or possibly by the same cells reacting in different fashions to different types of stimulus.

CHAPTER II

THE FUNCTIONS OF PLASMA PROTEINS

ANTIBODY, at least of the classical types, is part of the plasma protein of the animal possessing it. It is therefore desirable to consider the general composition and functions of the plasma proteins as a background to more detailed discussion of the antibodies themselves.

Mammalian plasma contains a very complex mixture of proteins. These have always been of the highest interest to physiologists and with each new development of physiological and biochemical techniques or interests, these have been applied to the plasma proteins. As a result growth of knowledge has been extensive but to a large extent uncoordinated, the findings by one particular group of techniques being sometimes completely unrelated to those by another. This has been partly remedied in recent years by work such as that under Cohn's direction in which relatively large teams of specialists have been concerned. In many respects it is possible to present a well integrated picture of the important protein components of plasma and their functions but in some equally important aspects, especially the mutual influence of the protein components on one another and their relation to lipids and polysaccharides, much remains to be learnt. There remain also a number of protein components present in small concentrations that are still measurable only by their biological activity (e.g. certain enzymes, hormones, complement etc.). With this qualification the functions of the plasma proteins other than those of immunological character can be considered under five headings.

(1) *The maintenance and stabilization of blood volume.* Since the time of Bayliss and Starling this has been regarded by physiologists interested in problems of the circulation as the principal function of the plasma proteins. The exchange of fluid between blood and tissues and the maintenance of blood volume depends upon the balance be-

tween the hydrostatic pressure of blood in the capillaries and the colloid osmotic pressure, due to the plasma proteins and more especially to the serum albumin. Albumin, with its relatively low molecular weight and high net negative charge at the pH of blood, is far more effective than any other plasma protein in maintaining the osmotic pressure. The low viscosity of albumin, due to the low asymmetry of its molecules, also fits it for this function. Albumin is probably produced in the liver (Madden and Whipple 1940).

(2) *The transport of hormones and enzymes.* Many hormones (e.g. the thyreotropic hormone) and enzymes occur in minute quantities in the blood stream.

(3) *The transport of lipids and other substances in close association with the plasma proteins.* Many biologically important lipids are transported in the plasma in close association with certain globulin components. In this way relatively high concentrations of materials which are virtually insoluble in water or aqueous salt solutions are held in stable aqueous solution in the plasma. As Bennhold (1932) first emphasized, serum albumin also shows specific powers of reversible combination with a large variety of molecules and serves to transport them through the plasma.

(4) *Protection against blood loss by the clotting mechanism.* An essential part of the mechanism of protection against blood loss following trauma is provided by the process of clot formation. Both fibrinogen which forms when converted to fibrin the actual substance of the clot and prothromin have been prepared as substantially pure proteins. There is good evidence that fibrinogen is formed in the liver.

(5) *Nutritive functions of the plasma proteins.* Investigations by Whipple and his associates (Madden and Whipple 1940) on dogs subjected to plasmaphoresis, and by Schoenheimer (1942) using isotopic markers, have shown conclusively that the plasma proteins, like the cellular proteins, are in constant state of flux, being rapidly degraded and resynthesized. Recently Fink and others (Fink, Ennis, Kimball, Silberstein, Bale, Madden and Whipple 1944) prepared labelled plasma proteins by feeding dogs

with lysine containing N^{15} and these labelled proteins were then injected into other dogs to replace plasma proteins removed by bleeding. In normal dogs half the labelled protein disappeared in 26 hours but the further loss of half the remaining material took five days. This was interpreted as indicating a mixing within one day of the injected proteins with a pool of mobile tissue proteins, which is about as large as the amount of plasma proteins in the blood stream. The subsequent loss of labelled protein is the result of degradation and resynthesis of the plasma proteins. These authors found the half-life time for circulating of proteins in dogs to be of the same magnitude as in rats (Schoenheimer, Ratner, Rittenberg, and Heidelberger 1942) and humans (Rittenberg and Shemin 1947).

ANTIBODY GLOBULIN AS PART OF THE BLOOD PROTEIN RESERVE

Investigations of the fate of antibody globulin in rabbits by similar techniques (Schoenheimer, Ratner, Rittenberg and Heidelberger 1942, Heidelberger, Treffers, Schoenheimer, Ratner and Rittenberg 1942) showed that in an actively immunized animal the antibody globulin incorporated dietary nitrogen at about the same rate as did the other plasma proteins, the half-life time of the antibody molecule being about two weeks. If immune serum from a rabbit which had been immunized with a type I pneumococcus was inoculated in a single large dose to a rabbit which had been actively immunized with type III pneumococcus and isotopic glycine administered, it was found that the type III (actively produced) antibody quickly took up N^{15} as before, but it was not incorporated into the injected type I antibody. The passively introduced antibody disappeared more rapidly than that actively produced, and the maintenance of the level of active antibody by its continual production *in vivo* was reflected in the fact that of the two antibodies circulating in the animal, only that produced by the tissues of the host contained dietary nitrogen.

Although the globulins have been shown to undergo

breakdown at the same rate as albumin and the tissue proteins, there is evidence that after severe nitrogen depletion, in both humans (famine oedema) and dogs (Zeldis, Alling, McCoord and Kulka 1945) the globulin content of the plasma remains essentially normal while the albumin is greatly decreased. Similarly, after plasmaphoresis experiments in which the tissue reserves and all plasma protein components are depleted globulin is always produced in greater relative proportion than its normal abundance in the plasma (Madden and Whipple 1940). Haemoglobin is even more rapidly and abundantly produced (Whipple and Madden 1944) and these differences may merely reflect the varying efficiency of the albumin-, globulin- and haemoglobin-forming cells in utilizing the available amino-acids for synthesis.

The percentage of protein in serum and its distribution between albumin and globulin fractions may vary within wide limits, particularly if pathological sera are included. In cases of multiple myeloma (Kekwick 1940) considerable anomalies are observed in electrophoretic tracings, and in nephrosis and famine oedema the albumin is greatly diminished compared with the globulins. An increase in α globulin has been found in all cases of infection in which there is tissue destruction or inflammation (Longsworth, Shedlovsky and McInnes 1939).

Hyperimmunization of horses (van der Scheer, Wyckoff and Clarke 1941, van der Scheer, Lagsdin and Wyckoff 1941) and of rabbits (van der Scheer, Bohnel, Clarke and Wyckoff 1942) causes great increases in globulin, usually in γ globulin but sometimes (antitoxins in horses) in the new T component. In rabbits with bleedings late in the course of hyperimmunization the increase in γ globulin was almost wholly accounted for by the increase of precipitable antibody, with earlier bleeds discrepancies occurred which may have been due to the production of normal globulin or perhaps of "univalent" or "degraded" non-precipitable antibody.

These findings suggest that a considerable proportion of the serum globulin is or may be produced by the same cells as are responsible for the production of antibody. In

a later section on the immunological behaviour of young animals, further evidence in this direction will be produced. Here we need only refer to the fact that γ globulin (and antibody) are completely absent from the new-born calf (before it has suckled) the principal globulin being fetuin which is probably an α globulin of very low molecular weight (Pedersen 1944-1945). With the ingestion of colostrum, antibody and γ globulin appear promptly in the calf's serum. In calves reared without colostrum, on the other hand, Hansen and Phillips (1947) found that the fetuin (α globulin) content gradually fell and γ globulin gradually rose until adult levels were reached about eight weeks after birth. It appears that there is a definite component of serum globulin not produced in the sheltered uterine environment, but necessary for protection against bacterial invasion (Smith and Little 1922a), and others which are so produced and therefore cannot be regarded as having only immunological functions.

CHAPTER III

THE RISE AND FALL OF ANTIBODY TITRE IN THE BLOOD OF ACTIVELY AND PASSIVELY IMMUNIZED ANIMALS

Following an adequate antigenic stimulus, which may be either a natural or artificial infection by living micro-organisms or the injection of some non-living antigen, the animal responds by the production of antibody. The concentration rises after a preliminary lag period to a maximal level, and then falls more slowly. The rate and extent of such changes vary widely with the nature of the antigenic stimulus, the species and past history of the animal, and its physiological state during the immunization period. There are, however, certain basic regularities in regard to the time relations of the antibody response, which form the subject matter of this section.

THE DIFFERENCE BETWEEN PRIMARY AND SECONDARY RESPONSE

When an animal is injected with an antigen which it has never before encountered, the antibody response is almost always less active than it is in an animal which has previously reacted to the same antigen. This difference between primary and secondary responses is particularly evident in experiments with bacterial toxins or toxoids. Glenny and his collaborators have studied the phenomena in various animal species immunized against diphtheria toxin, and a review of the literature will be found in Glenny's (1931) article. The same difference in response is well shown with injections of staphylococcal toxoid, and in 1931 one of us (F.M.B.) carried out a detailed study of the characteristics of the responses to this antigen. Staphylococcal toxin is particularly suitable for work of this sort, since the antitoxin can be titrated to any desired degree of accuracy by a simple *in vitro* technique. Two features of the results were of particular interest. In the

first place a primary intravenous injection of toxoid produced practically no formation of antitoxin although it rendered the animal capable of giving a typical sharp secondary response to the next injection. Subcutaneous injections of toxin or toxoid, on the other hand, produced a slow but considerable appearance of antitoxin after the initial injection. The second feature was the way in which the increase of antitoxin during the period from 48 to 96 hours after a secondary intravenous injection of toxoid appeared to follow a strictly logarithmic course. A brief account of these experiments, which have been previously published only in the first edition of this monograph, is included here to supply a basis for comparison with the phenomena encountered with other types of antigen.

(a) *The Primary and Secondary Responses to Staphylococcal Toxoid*

Technique: The experiments reported were carried out in 1931 using the technique described in earlier papers (Burnet 1929, 1931). The material used for primary inoculations was either toxin or formol toxoid, given by various routes, but all the secondary responses shown were obtained following intravenous injection of formol toxoid, i.e., "soft agar" toxin rendered non-toxic by incubation overnight with 0.4 per cent formaldehyde. In two experiments purified high titre toxoid, made as described by Burnet and Freeman (1932), was used for the secondary stimulus.

Antitoxin titrations were made in terms of a local provisional standard by the haemolytic method, and taking the end point of complete absence of haemolysis after the tubes had been held one hour at 37°C., and overnight in the refrigerator. The unit used is approximately 1/125 of the International unit.

In all experiments blood samples were taken from the ear vein, the serum separated and stored in capped tubes in the refrigerator till the series of bleedings had been completed. Rough titrations were made using doubling dilutions of serum, and when the approximate titre had been determined an accurate titration to within 10 per

cent was carried out. These final titrations were all done at the same time and against a single batch of toxin, so that the titres shown at different periods by the one rabbit are strictly comparable.

Provided one is careful to avoid naturally immune rabbits by a preliminary titration of the serum, the difference between the primary and secondary responses to toxin or

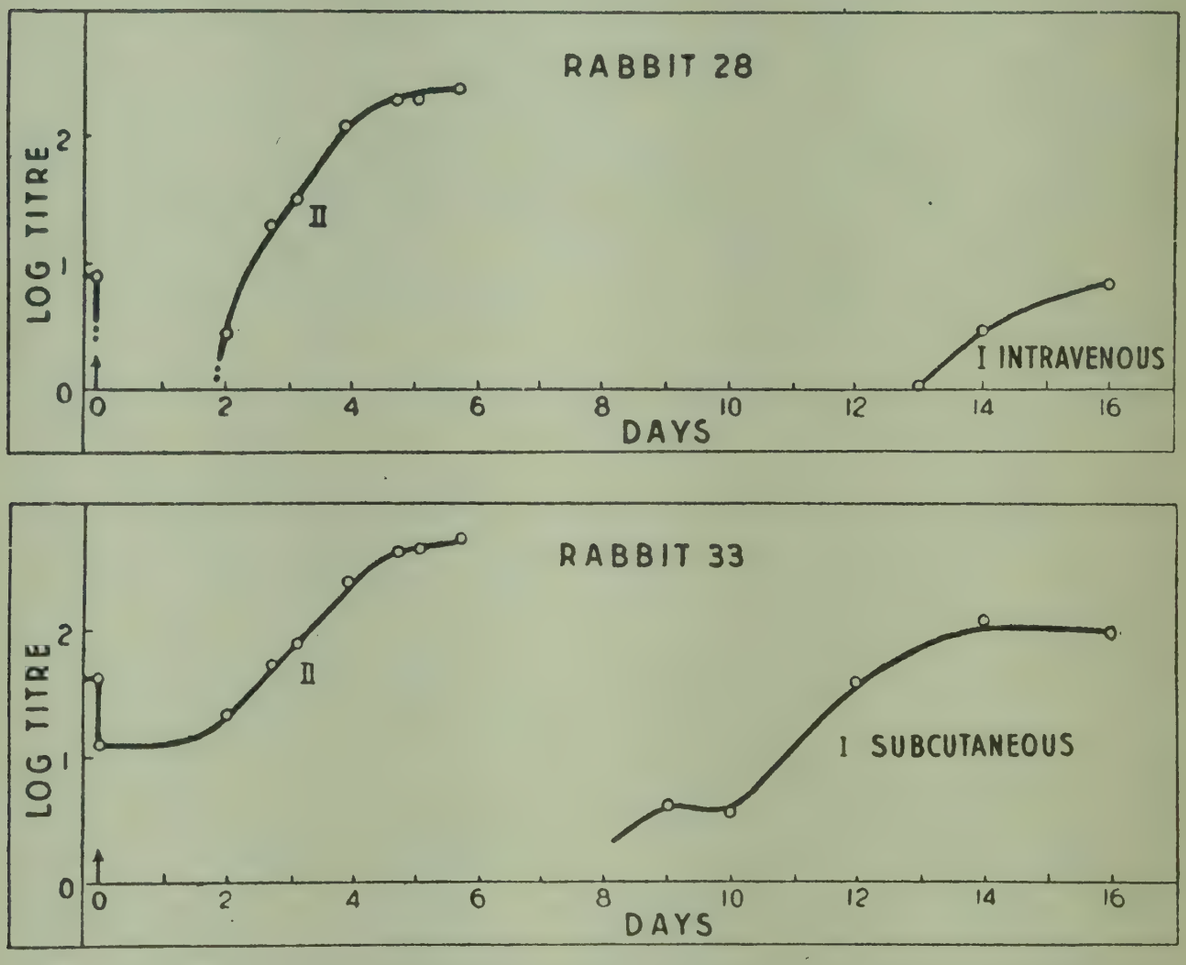


FIG. 1. *The primary and secondary antitoxic responses of two rabbits to staphylococcal toxoid.* Both primary and secondary injections were given at the point 0. Curve I is the slowly appearing primary response, Curve II the rapid secondary response to intravenous injection made eighteen days after the primary stimulus.

toxoid is very clearly shown. The primary response is more evident with subcutaneous than with intravenous injection, but in either case is slight and relatively slow. In none of the animals tested was any antitoxin present before the seventh day, and the maximal titre was reached in from ten to fourteen days.

Once a basic immunity had been established the response to further injection of toxoid was much more rapid and followed a very regular course. Immediately following

the intravenous injection there was the expected fall of antitoxic titre and no definite increase until about 40-48 hours after the injection. For the next 48 hours there is an accelerating increase in titre, and then a slower increase for one or two days, the peak occurring on the fifth or sixth day.

In Figure 1 the primary and secondary responses of two rabbits are shown. These are quite typical of the group of eight used in this experiment, four being given the

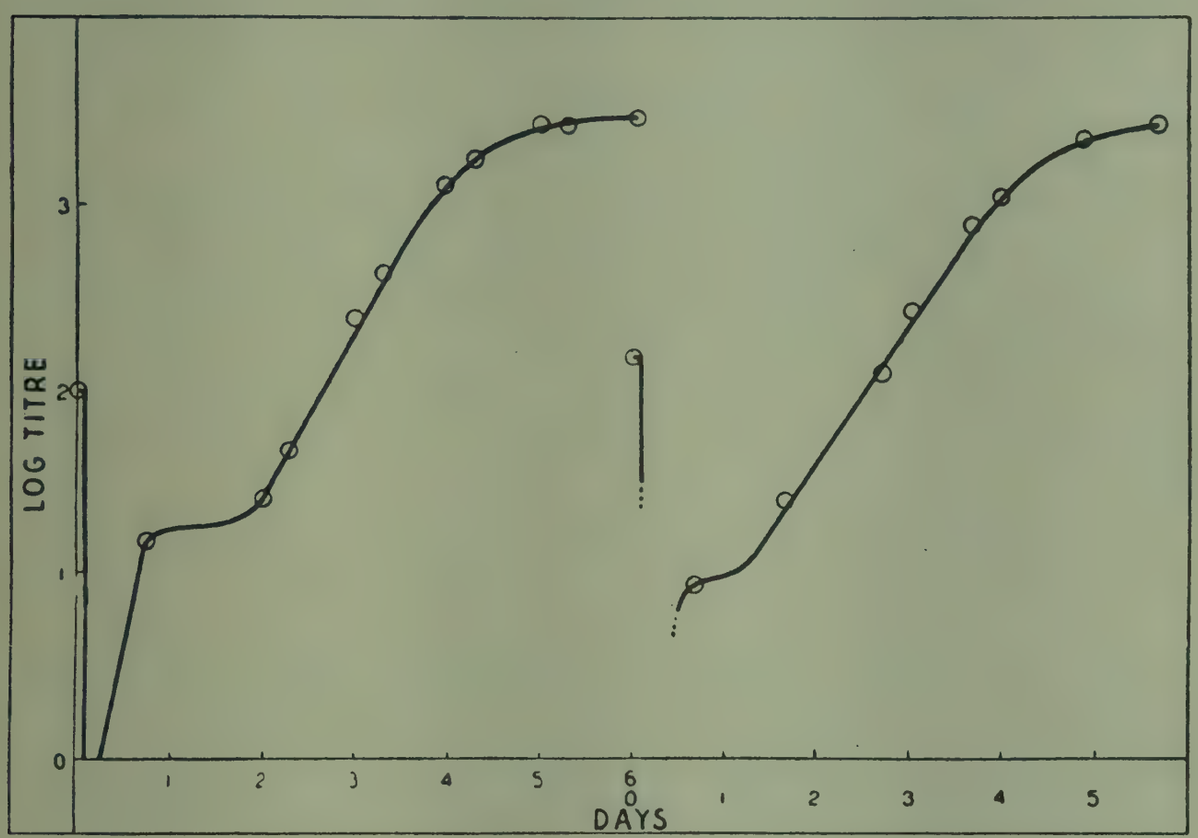


FIG. 2. *The secondary responses of two immune rabbits to a single very large intravenous dose of concentrated toxoid.*

primary injection of toxoid subcutaneously and four intravenously. In all cases the second (intravenous) injection was given eighteen days after the primary injection. The difference in the primary response to intravenous and subcutaneous inoculation shown with these rabbits was also seen in the others of the group, and there is a suggestion from the curve that the later part of the rise in titre of the subcutaneously injected rabbits is essentially a secondary response to antigen which is being slowly absorbed from the local deposit. The most interesting feature of the secondary curves is the occurrence of a "logarithmic

phase" of increase in the period from 48 to 96 hours. This type of secondary response was obtained in all of eleven rabbits in which detailed titrations were made. Where the intravenous injection of toxoid resulted in the temporary complete disappearance of antitoxin the earlier part of the logarithmic phase is distorted, but in more substantially immune animals there is a very close approach to a straight line over this portion of the curve of antitoxic titre. This is particularly well shown with two previously immunized rabbits which were given very large intravenous doses of formol toxoid made from purified and concentrated toxin. The vigorous antitoxic response to this treatment is shown in Figure 2.

Examination of the whole series of curves obtained during these experiments indicates that the slope of the logarithmic portion appears to be related to the degree by which the initial titre was lowered by the intravenous injection. The greater the fall the steeper is the slope of antitoxin increase.

(b) *Primary and Secondary Responses to Particulate Antigens*

(i) Bacteria: When the particulate micro-organismal antigens are studied in the same manner there is by no means such a sharp difference between primary and secondary responses. If a rabbit is inoculated with a single moderately large dose of a bacterial vaccine, agglutinin appears in the serum about the fifth day and reaches a high titre in twelve to fourteen days. The secondary response may reach its peak a day or two earlier than that following a similar primary stimulus, but the difference is in no way comparable to that observed with bacterial toxoids except when very small doses of antigen are used. In Topley's (1930) experiments a primary intravenous dose of 10^4 killed paratyphoid bacilli produced no agglutinin, but subsequent doses of the same amount provoked a definite amount of agglutinin in three of four rabbits. An important difference between primary and secondary responses to *B. typhosus* vaccines discussed by Topley (1933) is the longer duration of circulating antibody found after secon-

dary inoculations. The fall in titre following the peak of the response is much slower, a finding that was also noted by Jensen (1933) in children immunized with diphtheria toxoid.

Rickettsiae, viruses and bacteriophages resemble bacteria rather than toxins in their behaviour as antigenic stimuli. Since no work on the antibody response to these antigens at all comparable to the detailed studies on antitoxins and bacterial agglutinins has been published, a series of experiments were carried out with typical members of each group in rabbits.

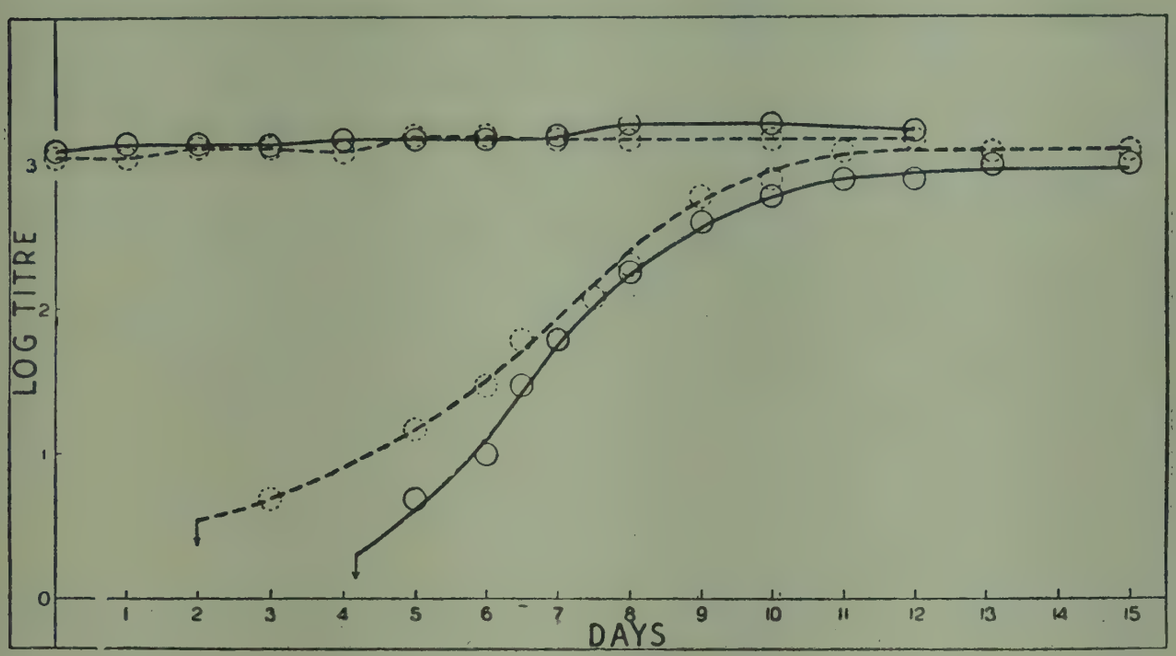


FIG. 3. *Primary and secondary response of two rabbits given rickettsial emulsion intravenously*. There is practically no secondary response in either animal.

(ii) RICKETTSIAE: A method of preparing a relatively pure suspension of the rickettsiae of Q fever from infected mouse spleens and livers has been described by Burnet and Freeman (1938). Such suspensions were used both as inoculum and as reagent for agglutination in the present experiments. Both primary and secondary inoculations were given intravenously, 2 c.c. of our standard suspension corresponding in opacity to a typhoid vaccine of about 500 million organisms per c.c. being used for each injection. Two rabbits gave approximately similar results, which are graphically represented in Figure 3. Agglutination tests were made with closely spaced dilutions of serum over the required range, the tubes held in a water bath at 45°C. for

six hours, and read after standing overnight at room temperature. The end point taken was a standard degree of granularity, values being interpolated between two tubes if necessary.

It will be seen that the rickettsial suspension is an excellent antigen and provokes an early primary response very similar to that given by bacterial vaccines.

(iii) Herpes Virus: Owing to the technical difficulties in preparing a purified virus antigen, these experiments were limited to following the appearance of antibody after corneal inoculation with a living non-encephalitogenic strain. In all probability no great absorption of virus antigen occurs until the keratitis is well developed on the third or fourth day, so that the real lag period is shorter than would be judged from the figure (Figure 4). The titrations were made by our standard chorioallantoic technique and the titres expressed in terms of the percentage reduction of specific foci by undiluted serum (Burnet, Keogh

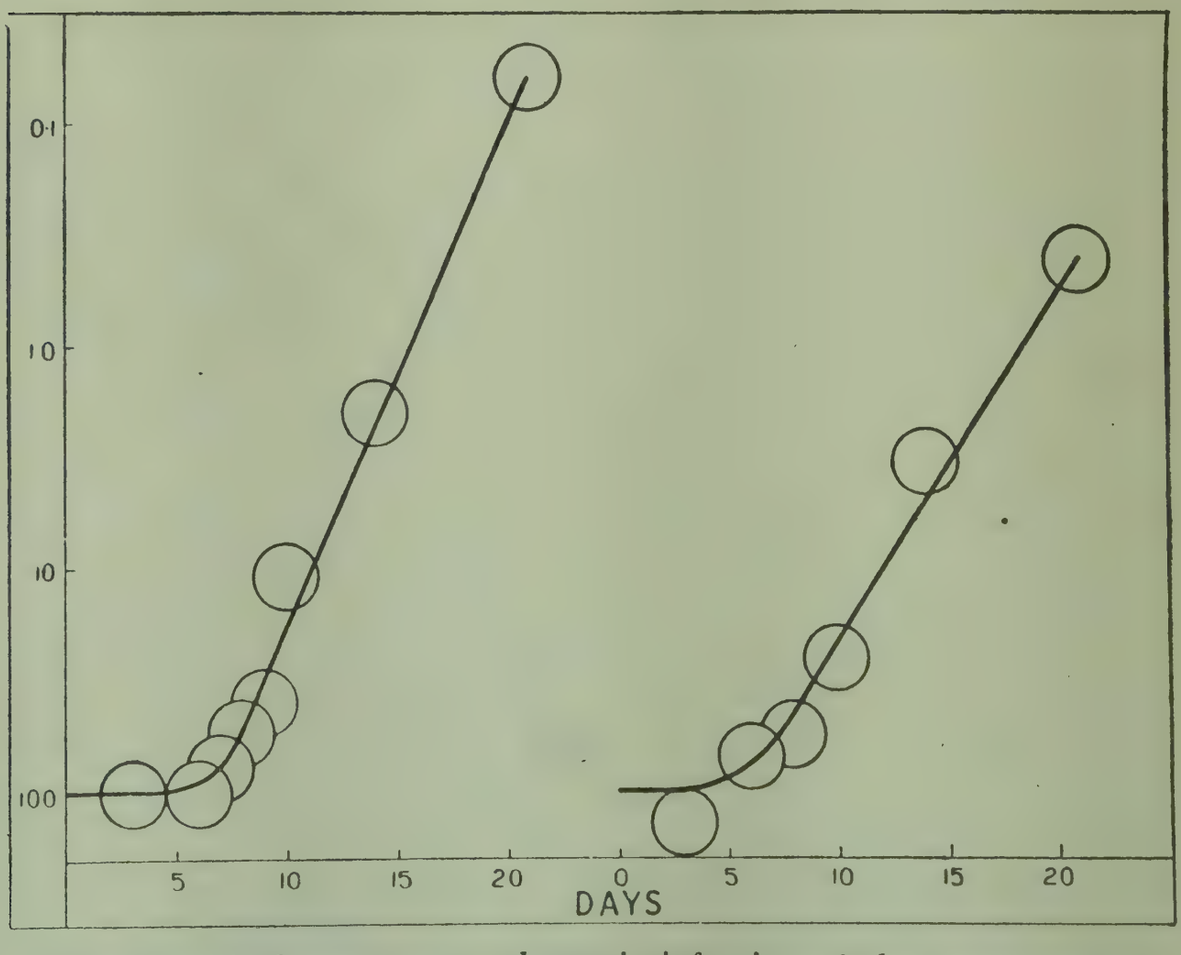


Fig. 4. *Antibody response to herpetic infection of the cornea in the rabbit.* Ordinates show the percentage of foci produced from virus serum mixtures on the chorioallantois.

and Lush, 1937). For obvious reasons no experiment on the secondary response could be made with this type of technique.

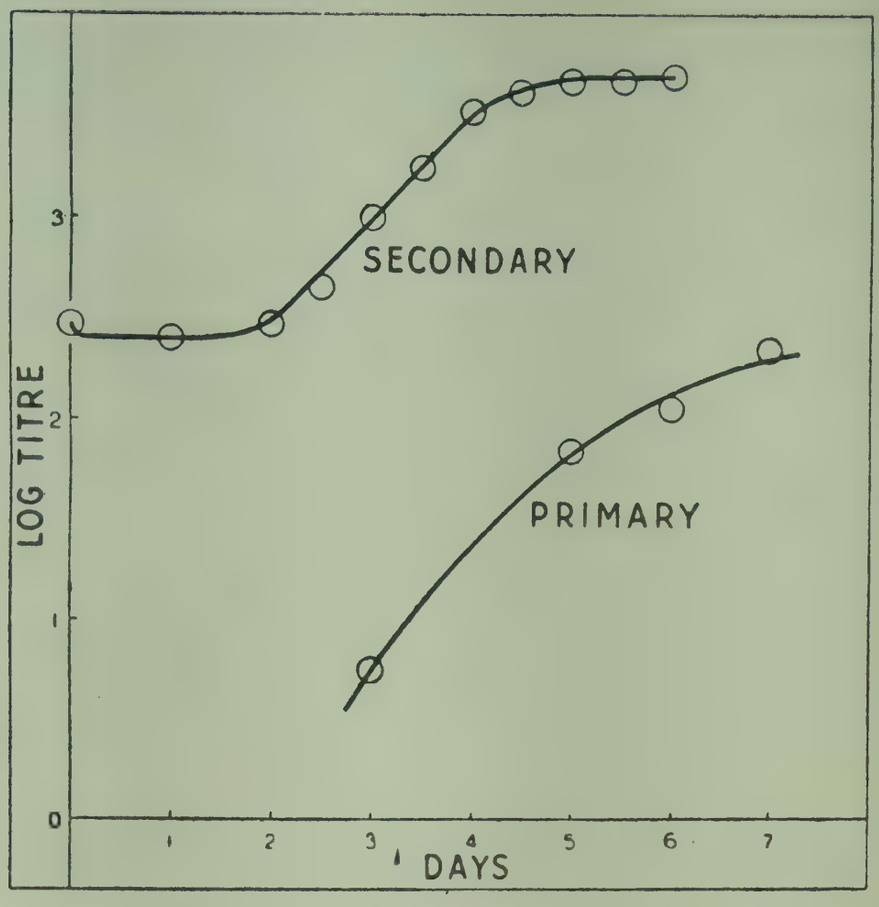
The results therefore merely confirm the findings of others that the antibody response to such viruses as vaccinia (Wilson Smith 1929), and influenza (Burnet and Lush 1938b) appears early and rises fairly rapidly.

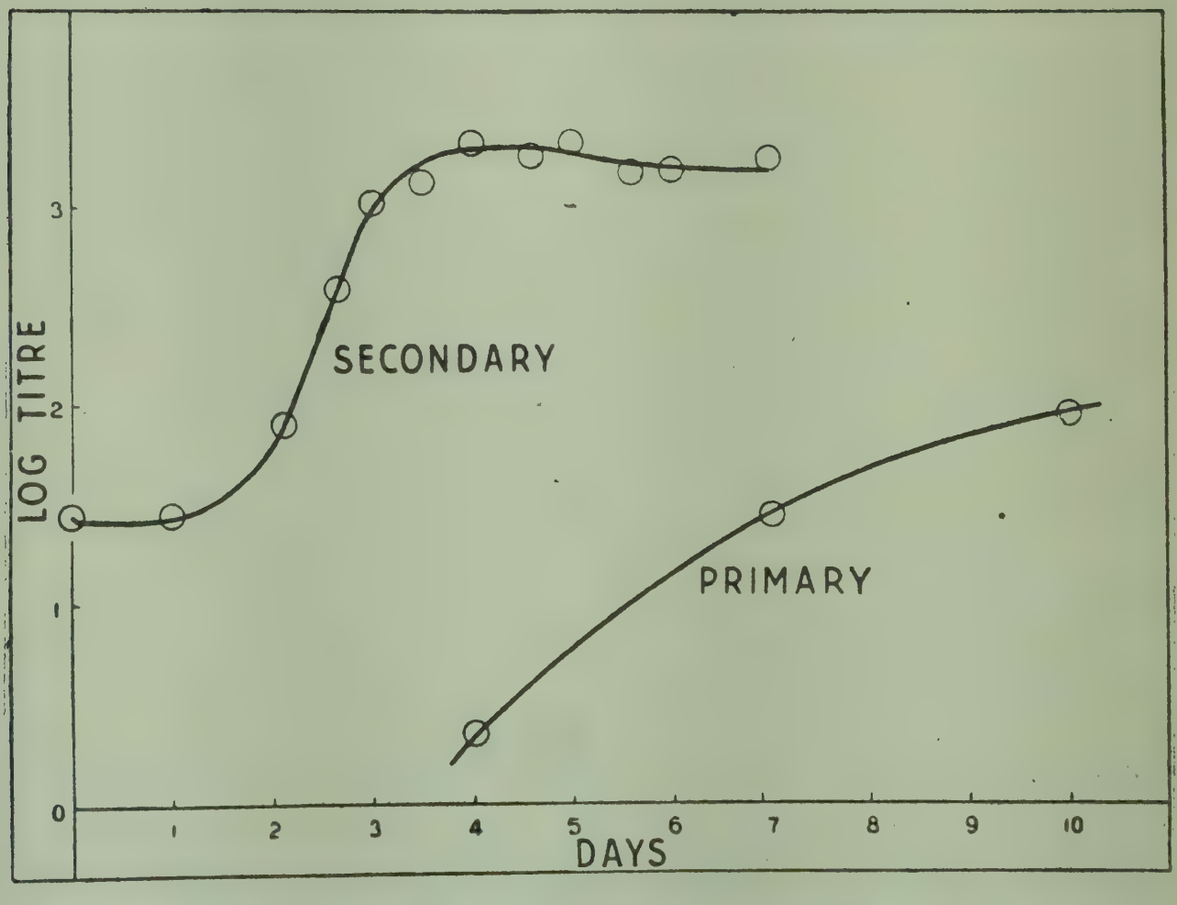
(iv) BACTERIOPHAGE: The well known phage C16 was chosen, since it is a potent antigen and can be titrated with considerable accuracy.

Technique: The phage was grown on a smooth strain of γ dysentery bacilli (Hiss and Russell) and filtered through a gradocol membrane. The stock phage had a titre of 2×10^9 particles per c.c. by plaque count titration on weak (1 per cent) nutrient agar.

Eight rabbits, all of which were shown by a preliminary test to possess no detectable antiphage, were inoculated intravenously with phage C16. Four were given 2 c.c. of undiluted phage, two were given a tenfold dilution (i.e., 4×10^8 phage particles) and two received phage diluted 1:100 (4×10^7 phage particles). They were then bled at three- to four-day intervals for three weeks. Two months later they were all given 2 c.c. of undiluted phage intravenously and were then bled twice daily for seven days. All sera were inactivated by heating to 56°C. for 20 minutes. The sera were titrated by the technique used in this laboratory (Burnet 1933). Briefly it is as follows:

A standard dilution of phage is so made up that 0.01 c.c. will produce about 150 plaques. Serial dilutions of serum, mixed with equal volumes of standard phage dilution, are heated in a water bath at 45°C. for one hour. They are then cooled on ice and 0.02 c.c. amounts plated out on 1 per cent nutrient agar previously spread with a few drops of a young broth culture of the susceptible strain. The plates are incubated overnight and the plaques counted next morning. The titre of the serum is expressed as the reciprocal of that dilution of serum required to reduce the plaque count to 20 per cent of the control mixture of standard phage dilution with an equal volume of broth.





FIGS. 5, 6. *The primary and secondary responses of two rabbits to intravenous injections of phage C16.*

All sera were titrated twice, first using tenfold dilutions of serum, and secondly using four doubling dilutions ranging round the approximate titre indicated by the first rough titration. Three plates were used for each phage-serum mixture, each series of three counts being then averaged. The serum titre (expressed as the logarithm of the titre as defined above) was obtained graphically by plotting the average percentage of plaques obtained against the logarithm of the corresponding serum dilutions. The point at which this line crossed the 20 per cent level was taken to indicate the logarithm of the serum dilution which would produce a reduction in plaque count to this figure. The experimental error was calculated by titrating one serum twelve times. The twelve results had a mean of 2.057, with a standard deviation of ± —0.03. This standard deviation was taken as a rough indication of the experimental error for the whole series. The titres were therefore plotted as circles, with a radius of twice the standard deviation (i.e., 0.06).

Figures 5 and 6 show two samples of the difference between primary and secondary antibody response.

Figure 7 shows the secondary response in the logarithmic phase (from second to fourth day) in four other rabbits.

When these curves are compared with those of the staphylococcal toxin series it will be seen that whereas

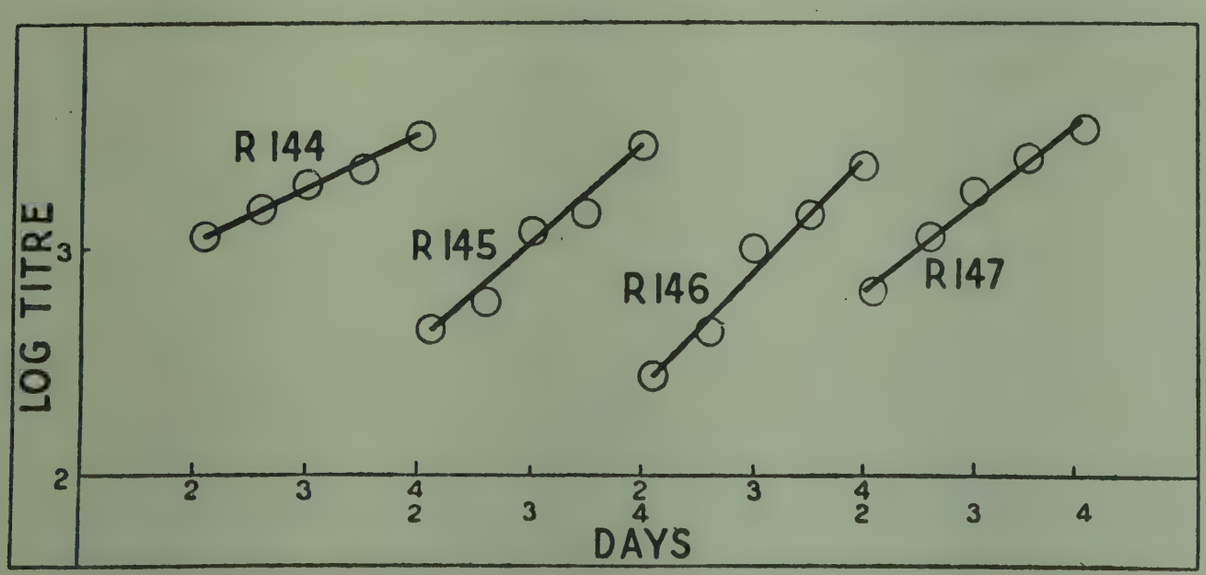


FIG. 7. *The "logarithmic" portion of the secondary response in four rabbits given phage C16.* In each case the portion of the curve from 48-96 hours after injection is shown.

the time of appearance and character of the secondary response are almost identical for the two antigens, there is a striking difference between the primary responses to intravenous inoculation, and shows a rapid and considerable rise in titre. This titre is well maintained over at least two months, and in some rabbits showed significant later rises. The cause of these has not been ascertained, but they may well have resulted from the establishment of the phage in the intestine with occasional leakage into the blood stream. The logarithmic character of the secondary response is not quite so exact as was observed with staphylococcal toxoid, but nearly all the curves show an approximation to a straight line over the two-day period in question.

THE CHARACTER OF THE PHASE OF ACTIVE ANTIBODY INCREASE

We are disposed to lay considerable stress on the regularity of the results obtained in studying the secondary response to a staphylococcal toxoid given intravenously. Relatively large amounts of a very active but non-toxic antigen, which is almost certainly in the form of a soluble protein of not very high molecular weight, were administered by a route which allowed rapid contact with the presumed antibody-producing mechanism. If there is an underlying regularity in the time relations of the antibody response it should be more easily visible in such a type of experiment than in any other way. All our experiments did in fact show a phase extending over at least 48 hours, in which the rise in antitoxin followed very accurately a logarithmic course. This is also more or less evident in the curves of antibody increase after injection of other antigens, but with these we can feel certain that the antigenic stimulus is not applied to the cells concerned in such simultaneous fashion as results from an intravenous injection of toxoid. Some degree of disintegration must probably be undergone by any particulate antigen before it can exert its full antigenic effect, so that the various phases of the response would tend to be blurred. With antigens injected by other routes than the intravenous one or enter-

ing the body as a living pathogenic micro-organism, the response would be still more obscured.

The form of the curve obtained in these experiments with a staphylococcal toxoid indicates that over a period of two days the rate of antibody production is proportional to the amount of antibody present in the blood at any instant. The suggestion is very strong that we are dealing with the *multiplication* of some entity concerned with antibody production. It cannot be a multiplication of antibody in the blood itself. Although at the time it was already well established that antibody production was a function of the tissues and not of the circulating blood, experiments were carried out to determine (a) whether normal rabbits given citrated blood from others in the "logarithmic phase" of antitoxin production showed a continuing rise, and (b) whether such blood caused a further response in basically immune resting rabbits. The experiments, as was to be expected, were completely negative. The only remaining hypothesis to account for the logarithmic increase is to postulate that over a short period something, somewhere, is proliferating in true biological fashion, and that this proliferation is reflected in the antitoxin increase.

There is evidence of another sort that a replication process is involved in antibody production, namely the lognormal distribution of titres obtained in any large series of animals uniformly exposed to an antigenic stimulus (Barr and Glenny 1945), (Carlinfanti and Cavalli 1945). As was pointed out earlier (Burnet 1946) the simplest explanation of this is probably that a replication process is at work, the extent of which is determined by factors which vary in a random linear fashion.

THE LATER PHASES OF THE ANTIBODY RESPONSE

By repeating inoculations during the period of rising titre, this stage can be prolonged for a few days, but the rise eventually ends, often rather abruptly, and is followed by a phase of diminishing titre. The duration of the period over which antibody remains above some standard titre is often a matter of practical importance, and a good

deal of rather uncoordinated data is available on the subject. Detailed information suitable for analysis from the theoretical point of view is, however, rare, and we shall make most use of the data in two papers, one by Jensen (1933) on the course of antitoxic titre following immunization of children with diphtheria toxoid, and the other by Mason, Dalling and Gordon (1930) on immunity against lamb dysentery toxin in sheep.

The fact that the "half-life" of antibody molecules in the plasma is only 10-14 days makes it obvious that when, as is usual, the initial response is followed by a slow fall in titre extending over months or years, production of antibody must be continuing at a slowly diminishing rate throughout the period. The observed curve of antibody titre then must be the resultant of two processes, the production of antibody and its destruction or removal from the circulation. It is, theoretically at least, possible to observe the second of these processes alone by following the rate of fall of antibody in a passively immunized animal. Unfortunately, various difficulties arise when we try to interpret the results and apply them to the processes going on in actively immunized animals. In the first place, many of the recorded experiments on the duration of passive immunity deal with the fate of an antibody derived from one species, usually the horse, when injected into a different species. Here the foreign nature of the protein carrying the antibody provokes an immunological response in the recipient, and after a week or thereabouts causes an acceleration of its removal. Even when serum from the same species is used there are possibilities that the antibody molecules have been altered during the clotting, separation and storage of the serum from the form in which they existed in the circulating blood. The experiments on passive immunity which seem to us least open to criticism are those of Mason, Dalling and Gordon (1930).

They studied the transfer of immunity and antibody from the ewe immunized during pregnancy to its lamb. This transfer is entirely by way of the colostrum, and takes place during the first two days of the lamb's life.

Ewes were immunized with formolized toxin or culture of the bacillus of lamb dysentery (*Cl. welchi*, Type B) during gestation. Immediately before parturition the ewe's serum and colostrum were titrated for antitoxin and afterwards repeated tests were made on the sera of ewe and lamb and on the milk for a period extending up to four months in some experiments. The antibody received by the lamb is of strictly homologous type, and for practical purposes can be regarded as being supplied only in the first two days of life, and predominantly in the lamb's first meal from the ewe's udder.

Figure 8 from Mason, Dalling and Gordon's paper shows the typical result of such an experiment. The antitoxic titre of the lamb's serum falls steadily, giving a time concentration curve which, within the limits of experimental error is a simple logarithmic one. A tenfold reduction of titre takes place in from 50-65 days in the three experiments whose details are available. In the same paper is the record of an experiment in which high titre diphtheria antitoxin (horse) was administered to the ewe shortly before parturition. Again the titres of both ewe and lamb were followed, but in this case both sera contained only passively given antitoxin, and that from a different animal species. In the ewe's blood the rate of fall was approximately logarithmic, and much more rapid than in the lamb, reduction to one-tenth occurring in about fourteen days, while the same proportionate reduction in the lamb took 48 days.

These experiments provide justification for considering that under ideal conditions passively administered antibody disappears from the circulation at a rate corresponding to the course of a monomolecular chemical reaction. This is, of course, the simplest *a priori* conception of the process, and involves no assumptions as to the actual mechanism by which antibody is destroyed.

Other studies on the disappearance of passively received homologous antibody give generally similar results, although some of the curves obtained depart somewhat from the simple logarithmic form. Neill and his collaborators (Neill, Gaspari, Richardson and Sugg 1932)

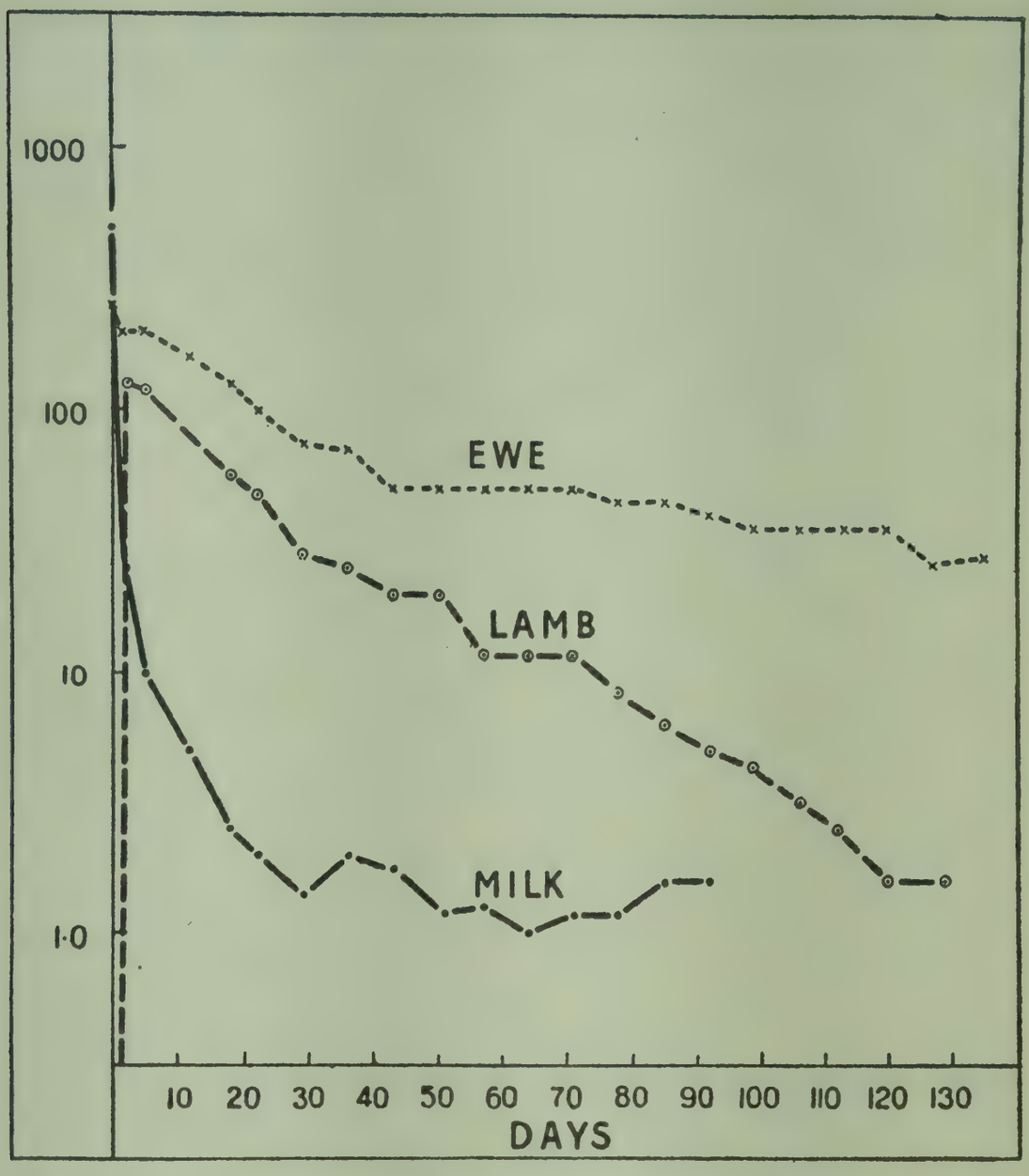


FIG. 8. *The concentration of antitoxin in the blood of a ewe immunized during pregnancy, in her colostrum or milk and in the blood of her lamb from birth onward.* Redrawn from Mason, Dalling and Gordon (1930).

found that the fall in diphtheria antitoxin in an infant whose mother's serum contained a very high level of antitoxin was logarithmic. In fifteen months the mother's titre only fell to 60 per cent of the peak titre, although she was apparently not a carrier of the organism, while the baby's titre fell to zero in about ten months. In Henderson Smith's (1907) paper the fall of agglutinin in a goat which received homologous immune serum subcutaneously is distinctly curved downwards when plotted logarithmically. The relatively rapid initial fall may be safely interpreted as resulting from the early saturation of the

recipient's tissue with antibody (cf. Sedallian, Jourdan and Clavel 1938, Freund 1927).

The conditions in regard to the fall in antibody titre after active immunization are naturally still more complicated. The curves obtained practically all resemble in essentials that taken from Mason *et al.* in Figure 8. The rate of fall is not logarithmic, but becomes slower with time.

The experiments of Schoenheimer, Heidelberger and their associates (1942) have been mentioned in the last chapter. Accurate quantitative estimations of the antibody content showed that in the rabbit 50 per cent of the homologous passive antibody had disappeared at the end of forty-eight hours, when the amount of active antibody had decreased by 28 per cent. N^{15} administered in dietary glycine was incorporated only into the actively produced and not into the passively administered antibody, indicating continuing production of the former during the period of decline of the antibody level. Study of the rate of decline of N^{15} in antibody after three days' feeding with labelled glycine showed that the half-life time of antibody nitrogen, (and therefore probably of antibody protein) in the actively produced antibody was two weeks. These experiments give an indication of the mode of destruction of antibody—it appears to be in the same dynamic state as the other plasma and tissue proteins and is constantly being utilized, probably after complete breakdown, as part of the body's reserve of labile protein.

Jensen's (1933) study of the antitoxin curves in children immunized against diphtheria by a single large injection of purified toxoid has provided some interesting results. Very great individual variations were observed in the maximal titre reached and in the period during which the antitoxin remained above the Schick level. Almost all the curves depicting the gradual fall of antitoxin once the peak titre had been passed could, however, be fitted to the formula for a bimolecular chemical reaction: $kt = \frac{x}{a(a-x)}$ where a is the maximal concentration of antitoxin and $(a-x)$ the concentration at the

time t after the time of highest concentration, and k an arbitrary constant. In two instances the reaction of the same child after primary and secondary injections was observed. In each case the second injection gave a more persistent rise than the first. The children were only observed for about a year, but using the bimolecular reaction formula, Jensen calculated that the duration of immunity above the Schick level would vary in different individuals from a few days to about 65 years. Differences of this extent in the response to a uniform antigenic stimulus must obviously be due more to variations in the response of the antibody-producing mechanism in different individuals than to any differences in the rate at which antibody is removed. Jensen found that the formula he used could also be applied to the results of animal experiments, such as those of Glenny and Südmersen (1921) who followed the antitoxin curve in several species immunized with diphtheria toxin-antitoxin mixtures. Madsen (1904) was also interested in the mathematical expression of the curve of antibody fall, but used a more generalized form, the rate being expressed as

$$\frac{dx}{dt} = k(a-x)^n$$

where n might have values from 1.2 to 3.5.

These expressions can have no real bearing on the processes concerned in the gradual diminution in antibody titre since they take no account of the continuing production that is known to occur. The most interesting feature of Jensen's results is the extreme variability in the persistence of antibody in different individuals. Such differences must in all probability be ascribed mainly to differences in the rate at which antibody production diminishes. In a later section evidence will be given that the acute response to an antigenic stimulus is associated with a characteristic plasma cell activity that lasts for only a few days to be followed by a phase in which the lymphocyte is the chief liberator of antibody. A reasonable assumption is that the rate of production of antibody falls off rapidly with the cessation of the plasma cell response and that the production rate by the maintenance (lym-

phocytic) mechanism diminishes in logarithmic fashion with time. This rate varies greatly from one individual to another and will be subject to modification by various physiological and pathological influences.

Freeman (unpublished) has followed in detail the fall of antibody titre in rabbits immunized (a) by two injections of staphylococcal toxoid, (b) by a single intravenous injection of phage C16 inactivated by formalin. Bleedings were made at frequent intervals during the early stages and later at approximately monthly intervals, the last being made from 137 to 145 days after immunization commenced. There was a very sharp difference between the two series, the staphylococcal antitoxin reaching its peak in five to six days after the second injection of antigen and falling along a curve corresponding as a rule fairly closely to Jensen's formula. Five of the six rabbits used showed a fall from the peak level to less than one-twelfth of this value by the end of the experiment. The remaining animal showed a rise in titre about a month after inoculation to a new and higher peak. This presumably represented the response to a staphylococcal infection of some sort, and as might be expected, the subsequent fall of titre to one-sixth of the peak level was less than in the other animals. The antibody of the rabbits immunized with phage C16, on the other hand, rose rapidly at first, but did not reach its peak until thirty to fifty days after inoculation. Three rabbits remained at this peak level without significant drop for the whole of the remaining three to four months, while the other three fell to one-half or one-third of the peak value.

The difference between the two series again draws attention to the contrast in behaviour of toxoid and particulate antigens. The phage experiments were made with formalin-inactivated material to ensure that the prolonged maintenance of titre we had previously noted was not due to the escape of active phage into the bowel and its continued multiplication at the expense of intestinal bacilli. The results seem to indicate that this particular phage is a very potent antigen, inducing a steady pro-

duction of antibody for long periods of time. It may perhaps be regarded as a "model" of the process by which an attack of measles or yellow fever gives rise to a life-long immunity.

MODIFICATION OF THE ANTIBODY CURVE BY AGENTS OTHER THAN SPECIFIC ANTIGENIC STIMULI

General experience teaches that the commonest and most potent stimulus in causing a rise in antibody titre is a new contact with the specific or a closely related antigen. In several instances a sharp rise in antitoxin was observed in the course of Jensen's studies on immunized children. These rises in antitoxin level may be safely attributed to subclinical infections with diphtheria bacilli. The toxin liberated in the course of such infections will naturally act as a secondary antigenic stimulus. There is a good deal of evidence that various forms of non-specific treatment can influence the antibody level of an animal, but the irregularity of the results makes one wonder whether in many instances technical flaws, particularly the inadvertent injection of small amounts of specific antigen, may not have been responsible.

The effect of bleeding has been investigated by many workers, and there is general agreement that repeated bleedings do not reduce the antibody titre by the amount which would be calculated from the volume of plasma removed. In a number of instances actual increase in titre has followed bleeding. Dreyer (Dreyer and Ainley Walker 1910), for instance, stated that "by repeated bleedings at the proper intervals one can keep up the percentage of antibodies in the blood long after it would normally have fallen to a low level, and can even cause an increase above the former maximum." Hartley (1924) obtained rises in titre in two of seven animals after repeated small bleedings. Mills (1925) studied the regeneration of antibody (haemolysin) in parallel with the regeneration of haemoglobin after bleeding. There was a certain degree of parallelism, but the antibody never showed as complete a recovery as the haemoglobin. O'Brien (1913) found that numerous large bleedings from a horse immun-

ized with sheep red blood cells had very little depressing effect on the antibody level. Commencing at a time when the haemolysin titre appeared to be stationary, he removed 122 litres of blood over a period of eleven months. After losing in this way several times its original volume of blood, the animal showed a drop in titre from 600 to 400 units only. Some authors, particularly Hahn and Langer (1917), have described enormous rises in antibody after repeated large bleedings in rabbits, but have not been able to particularize the conditions of their experiments sufficiently to allow others to confirm their results.

Somewhat similar evidence of continuing antibody formation may be obtained by following the diphtheria antitoxin titre through the life of a small animal. Glenny and Südmersen (1921), for instance, describe a guinea pig inoculated in November, 1911, when it was presumably of about 250 gm. weight; at the end of January it weighed 570 gm. and had an antitoxic titre of 0.14 A.U.; it remained at this level till November, 1912, when it weighed 935 gm., and in August, 1913, still had more than 0.09 A.U. During the period it had given birth to eight young and had increased greatly in weight. Another guinea pig showed no change in its antitoxin level of 0.15 A.U. from four to sixteen months of age.

There appear to be very many substances of the most diverse character which, on injection into an animal fairly recently immunized *may* produce a rise in titre of the original antibody. The lists include foreign proteins and heterologous bacteria, proteoses, sodium nucleinate, colloidal metals and various metallic salts, particularly of manganese and beryllium. Probably the best substantiated of all agents was manganous chloride, which, according to Walbum and Mörch (1923) had a striking effect in increasing the concentration of antitoxin in immunized animals, yet their results could not be confirmed by O'Brien (1924), or by McIntosh and Kingsbury (1924). The latter workers suggest the possibility that Walbum and Mörch's results may have been obtained only from horses whose antibody-producing mechanism had been "exhausted" by repeated immunizations. Mackie (1925) fol-

lowed the level of a "normal" antibody (anti-sheep cell haemolysin) in the rabbit under the influence of various non-specific injections of the type under discussion. The results were extremely variable, but about a third of the animals showed significant but not very large rises in titre. The only non-specific agents capable of giving a sharp increase in titre were those known to contain heterophil (Forssman) antigen, e.g., Shiga dysentery bacillus vaccines. Dreyer and Ainley Walker (1910) showed that significant rises of *E. coli* agglutinin could be produced in rabbits by inoculation of staphylococcal vaccine provided the second inoculation was made not too long after the primary immunization with *E. coli.*

Chase, White and Dougherty (1946) showed that adrenal cortical extract, inoculated along with an antigen, resulted in a considerably increased antibody titre in the mice, rats and rabbits that they tested. In hyperimmunized rabbits a single subcutaneous inoculation of adrenal cortical extract produced a sharp increase in circulating antibody six to twelve hours later, followed by a fall to normal after 25 hours. They attribute this to increased rate of release of antibody globulin from lymphocytes by the increased amounts of adrenal cortical hormone. White and Dougherty (1946) consider that most of the increases in antibody titre following diverse non-specific stimuli are due to the production and liberation of this adrenal cortical hormone, which causes greatly increased "dissolution" of lymphocytes and consequent release of antibody. Robertson (1948) is highly critical of the evidence presented by White and Dougherty for the occurrence of such dissolution.

There is a considerable amount of concordant evidence that tuberculous infection in rabbits and guinea pigs is associated with a non-specific increase in the ability of the animal to produce antibody. Thompson (1922) found that the injection of tuberculin in rabbits one to two days before the injection of a single dose of sheep erythrocytes greatly increased the production of haemolysin. Lewis and Loomis (1924), using the same antigen, found more than ten times as much haemolysin was produced in tubercu-

lous as in normal animals. Lurie (1939) provides evidence of a generally increased physiological activity of the reticulo-endothelial system in tuberculous rabbits and guinea pigs, and also in animals vaccinated with products of the tubercle bacillus. The cellular response to a non-specific inflammation was accelerated, the mononuclear phagocytes were larger, contained more vacuoles and showed a higher rate of multiplication than in normal animals. Their phagocytic capacity both for tubercle bacilli and for non-specific particles (carbon and staphylococci) was also increased.

"Blockade" experiments in which large doses of such substances as Indian ink, colloidal iron, and trypan blue are given, with the object of temporarily inactivating the cells of the reticulo-endothelial system, have given similarly variable results. Such experiments have been reviewed by Jungeblut (1930) and Jaffe (1931), and since very little of importance seems to have been done in this field since the appearance of these reviews, no attempt will be made to discuss the work in detail. Having regard to the high regenerative capacity of the cells concerned, the necessary irregularities in the proportion of cells blocked and the general toxic effect of the materials used, the results are compatible with the current view that the cells of the reticulo-endothelial system are responsible for antibody production, although they certainly cannot be held to prove this view.

There have been several investigations in recent years into the effect of nutritional deficiencies in diminishing the antibody response of the test animals compared with normal controls. Cannon and his associates (Cannon, Chase and Wissler 1943, Wissler, Woolridge, Steffee and Cannon 1946) have shown that if rabbits are subjected to plasmaphoresis while on a low protein diet, or when rats are kept for many weeks on a low protein diet, the ability to form antibodies is greatly impaired, but can be rapidly restored by feeding a high protein diet. They suggest that the results of Kligler, Guggenheim and Henig (1945) and Berry, David and Spies (1945) showing reduced antibody production by vitamin or protein deficient rats were

due primarily to lack of protein or interference with protein metabolism. However, Stoerk and his colleagues (Stoerk, Eisen and John 1947) found that pyridoxine deficiency had a striking and specific effect in impairing the antibody responses of rats, and they related this to the profound loss of thymic and lymphoid tissue that occurred in these animals. Since purified antibody globulin has been shown to contain several essential amino-acids, it is not surprising that gross dietary protein deficiency, or the lack of substances intimately concerned with protein synthesis (such as pyridoxine) should interfere with antibody production as well as the synthesis of other proteins.

COMMENT

In the first edition of this monograph the logarithmic rise in antitoxin production following the secondary stimulus of an intravenous injection of staphylococcal toxoid was interpreted as resulting from a phase in which the antibody-producing units were multiplying at a relatively constant speed somewhere within the body. It was in fact the form of these antitoxin responses that was responsible for the first development of the general theory presented. We are still inclined to the view that this is the most likely interpretation though we would agree with Northrop (1948) that the shape of the curves in themselves is inadequate evidence for such a conclusion. It must be stressed that there has never been any suggestion that antibody "multiplication" takes place in the circulating plasma. Our hypothesis is that when an antibody-producing mechanism has been established in a large number of cells by the primary stimulus, effective simultaneous contact of these cells with the toxoid given as a secondary stimulus results in a proliferation of the antibody-producing mechanism over a period of two or three days so that a progressively increasing number of units comes into action. The liberation of antibody into the blood parallels, presumably with a few hours' lag, the increase in the number of units producing it. From the histological evidence cited later in Chapter VI it seems probable that proliferation involves both cells and intracellular units.

CHAPTER IV

QUALITATIVE DIFFERENCES AMONGST ANTIBODY MOLECULES

Reference has already been made to the fact that the physical character of antibody molecules as judged by their electrophoretic behaviour may differ according to the species of animal or the antigenic stimulus used. In addition to these physical differences detailed immunological study will generally show that the population of specifically reactive antibody molecules in a given immune serum is not homogeneous and that successive samples of serum taken from one animal during different phases of an immunization procedure differ in the quality as well as the amount of antibody present. These qualitative differences amongst antibody molecules may be demonstrated by four types of technique.

(1) By absorption of the serum with a serologically related but not identical antigen. In general, if a serum anti-A is capable of reacting with a different antigen B, absorption of the serum with A will remove all antibody reacting with both A and B. Absorption with B will remove all activity against B, but activity against A will remain, in many cases with hardly any demonstrable change. Examples which may be cited are the synthetic glucoside antigens of Avery, Goebel and Babers (1932) and the egg albumin, dye-egg albumin systems of Heidelberger and Kendall (1934). The results obtained suggest that the degree of specific modification varies from one molecule to another, and that the specific pattern is not absolutely uniform. In a proportion of the antibody molecules it varies sufficiently in one direction from the norm to allow specific union with the heterologous antigen as well as with the homologous, and it is these variant molecules which are removed by absorption with the heterologous antigen.

(2) By quantitative studies of the combining power of

antibody with antigen along the lines developed by Heidelberger and his associates. In such studies as those of Heidelberger and Kendall (1935) serum samples are compared in regard to the amount of specific precipitate produced and the proportions of antigen and antibody in the precipitate, over a graded range of antigen and immune serum concentrations. The pattern of results obtained may vary significantly according to the stage of immunization of the animal.

(3) By titration of the same antibody type using different techniques. The type-specific antibody obtained by immunization with pneumococci can be recognized as a precipitin (with the polysaccharide), an agglutinin or as the mouse-protecting antibody. It was shown by Avery and Goebel (1933) that absorption with the original S.S.S. polysaccharide preparations did not remove the bulk of the mouse-protective antibody, but that the more complete acetyl polysaccharide would do so. Goodner and Horsfall (1937) found evidence that in horse anti-pneumococcal serum there exist at least three types of specific antibody with varying ratios between precipitating and mouse-protective power, while in the rabbit-immune sera only two components were recognized. One of these contained greater portion of the precipitin and showed a higher ratio of mouse-protective power to precipitating ability than the other. Heidelberger and Kabat (1936) found in concentrated horse-immune sera a good deal of type-specific antibody precipitable as agglutinin on the bacterial surface, but not by the polysaccharide.

(4) By inhibition tests. It has become evident, especially in connection with Rh blood group studies but also with bacterial antigens that, in addition to typical agglutinating antibodies, low-grade antibody may be developed which can be recognized either by the inhibition of the agglutinating antibodies, by techniques which indicate that the antigen is coated with low-grade antibody (e.g. Coombs, Mourant and Race 1945), or by agglutination tests carried out with a viscous suspending medium.

There is fairly conclusive evidence that an antibody molecule cannot function as such against two different

antigens. Dean, Taylor and Adair (1935) immunized rabbits with two antigens, egg albumin and serum albumin. The antisera produced by this double immunization behaved precisely as a mixture of two distinct antibodies, and there was no indication whatever of the existence of antibody molecules carrying modified areas corresponding to both antigens. It seemed possible that if one could ensure that two antigens were always taken up simultaneously by the antibody-producing cells, a detectable proportion of antibody molecules capable of reacting with both antigens might be produced. Freeman (unpublished) has carried out such experiments, using as antigen dysentery bacilli coated by Burnet's method with phage C16 and killed with formalin. In this way two totally distinct antigens are introduced into the cells in the form of a single particle. Both types of antibody were produced in large amount. The agglutinin was completely absorbed from the serum with homologous bacteria. The packed sediment of agglutinated bacteria was washed four times in saline and then treated with alkali by the method of Chow and Wu (1937) to elute portion of the absorbed antibody. The absorbed serum and eluate and a control eluate prepared from a pure antibacterial serum were tested for agglutinin and for antiphage according to standard techniques. The results were clear-cut in indicating (i) that all antiphage activity remained in the absorbed serum, (ii) that only a trace of antiphage activity was present in the eluate, and (iii) that this activity could be wholly ascribed to the liberation of small amounts of phage-inactivating bacterial polysaccharide (Burnet 1934) by the alkali used in the elution. Exactly similar results were obtained with a strain of *B. gallinarum* coated with the phage S1.

Information in regard to differences in antibody type either in regard to physical properties or immunological specificity is by no means systematic. It has to be drawn mainly from experiments carried out for other purposes and using a wide variety of antigens ranging from living bacteria or viruses to purified and semisynthetic antigens. At least four factors seem to be concerned—

(1) *The animal species injected.* In man and in the rabbit practically all antibodies, whatever the antigen and whatever the route of inoculation, are of the same type, namely γ globulin with a molecular weight of about 160,000. In the horse, on the other hand, most antibodies are of high molecular weight (about 1,000,000), but may migrate with either the γ or T globulins, and low molecular weight antibody also occurs. The latter also may migrate with the γ or the T globulins. In the cow and the pig high molecular weight antibodies of at least two electrophoretic types are produced, and perhaps smaller molecules also.

(2) *The route of injection.* In the horse, in which several different types of antibody molecule occur, the most important factor in deciding the nature of the antibody appears to be the route of inoculation (Treffers, Heidelberger and Freund 1947). Intravenous inoculation usually results in the production of no detectable antibody when diphtheria or tetanus toxin is used as the antigen or of high molecular weight antibody which migrates usually with γ globulin. Subcutaneous or intramuscular inoculation, on the other hand, is usually followed by the production of high molecular weight antibody with the electrophoretic mobility of the T component, although sometimes low-grade "univalent" antibody is also produced. In the rabbit, with the exception of certain haemolysins, antibody appears to be of only one qualitative type but the specificity of the antibody produced depends to a large extent on the route of inoculation. Intradermal or subcutaneous inoculation of pneumococci (Julianelle 1930) and of streptococci (Seegal, Heidelberger and Just 1934) results mainly in the production of species-specific antinucleoprotein, but intravenous injection of these organisms is followed by the abundant production of type-specific antibodies.

(3) *The nature of the antigen.* While the route of inoculation is apparently the most important factor in deciding the type of antibody produced by the horse, the nature of the antigen also plays a part, for as a rule anticarbohydrates are produced easily by intravenous inoculation and antiprotein antibodies (e.g. antitoxins) only by sub-

cutaneous or intramuscular injection. Further, while the subcutaneous inoculation of rabbit serum albumin gives rise to "flocculating" antibody, the inoculation of rabbit serum globulin by the same route produces only univalent antibody.

(4) *The duration of immunization.* When injections of the same antigen are repeated, systematic changes in the character of the antibody produced may be observed. In general the response to a single injection is to produce more specific antibody than is found when the animal receives a series of repeated injections. Studies on the antigenic relationships of serum proteins from a different mammalian species provide suitable material for demonstrating this difference. Wolfe (1935, 1936) found that a short series of three injections given on alternate days provided more specific sera for this purpose than could be obtained from rabbits reinoculated over a long period. As an example, antisera prepared against fitch serum by the two processes gave the following percentage activity against sera from other carnivores:

Short immunization—fitch 100, racoon 100, badger 1.5, skunk 1.5, dog 5.

Long immunization—fitch 100, racoon 100, badger 100, skunk 100, dog 37.

Similar results were obtained by Satoh (1933). Wolfe (1936) notes that, in addition to diminished specificity, antisera obtained after prolonged immunization produced more compact precipitates that settled rapidly to the bottom of the tube.

An example concerned with a series of related viruses is to be found in the papers by Francis and Magill (1938) on the serological classification of influenza virus strains. Serum neutralization tests were found to be more specific with serum from rabbits given a single immunizing injection than with that resulting from repeated injections. Similarly it was found that with repeated immunizing injections of virus intraperitoneally in mice their resistance to infection with heterologous strains broadened considerably.

Repeated reinoculations of the same antigen do not,

however, always result in antisera of diminishing specificity. In experiments with rabbits immunized with phage C16 it was found that the relative inactivating effect against related but not identical phages tended to become less with continued immunization (Freeman—unpublished), i.e., the antibody is more specific than that produced by a single injection.

Another type of difference was observed by Heidelberger and Kendall (1935) in rabbits immunized with crystalline egg albumin. They were concerned with the quantitative relations between the amounts of antibody protein and egg albumin precipitated under standard conditions. In one series they used three samples of serum taken from a rabbit at different stages in the course of a long series of immunizing injections. They found a regular change in the ratio of egg albumin nitrogen to antibody nitrogen in the precipitate, which could best be interpreted as being due to the formation of more and more antibody capable of reacting with a larger number of chemically different groupings in the antigen molecule. Another point of interest in their results was that in all sera, even those resulting from prolonged immunization, there is a proportion of low-grade antibody incapable by itself of forming precipitates and apparently consisting of globulin molecules with very few or only one combining group.

Certain antibodies are relatively resistant to pepsin and trypsin. By careful digestion with pepsin it is possible to reduce the average size of the molecules composing diphtheria antitoxin to a considerable degree without diminution in the antitoxic activity (Pappenheimer and Robinson 1937, Pope 1938). This insusceptibility to pepsin varies from one type of antibody to another, e.g., the O agglutinin for *B. typhosus* is consistently susceptible to inactivation with pepsin, while the H agglutinin may be highly resistant. While investigating this phenomenon, Rosenheim (1937) found that H agglutinin produced early in the course of immunization of horses was readily destroyed by pepsin, trypsin and activated papain. The antibody in sera from horses having had a prolonged course of immu-

nization had, however, become highly resistant to pepsin and trypsin, but not to papain.

Finally, we may mention the finding of Kabat (1939) that in the serum from horses which had been immunized against pneumococci for a considerable time a small proportion of antibody was present in smaller molecules. This "possibly degraded" antibody was not present in earlier bleeds from the same horses.

The various qualitative differences amongst antibody molecules which have been cited should serve to emphasize the characteristically biological complexity of the processes of antibody production. The findings in regard to the development of hypersensitivity to be discussed in a later chapter, strongly reinforce this impression. There is an implicit tendency to regard antibody as an almost automatically produced complementary equivalent to the antigen but in our view the *differences* amongst antibody molecules produced in response to the same antigen and especially the changes seen in the course of a prolonged series of antigenic injections are of special importance for any attempt to formulate the nature of antibody production.

CHAPTER V

THE SITE OF ANTIBODY PRODUCTION

Until very recently there was general agreement that the most likely producers of antibody were the cells of the reticulo-endothelial system. Two or three years ago the hypothesis that the lymphocyte was the transporter and perhaps the producer of antibody received strong support, while at the time of writing (1948) the chief interest is probably centred on the plasma cell. Recent authors who have been concerned to establish the importance of lymphocyte or plasma cell have however generally accepted the part played by the macrophage in initiating the process that culminates in antibody production. In our opinion the production of antibody is not a function of a single cell type but a complex activity of more than one type of cell. It takes place characteristically in situations where phagocytic cells of the reticulo-endothelial system are associated with lymphocytes and undifferentiated mesenchymal cells. Most of the discussion will be concerned with the respective roles of these cells and it is convenient to approach it by an examination of the function of the cells of the reticulo-endothelial system.

THE RETICULO-ENDOTHELIAL SYSTEM

There are two important and related functions for which the "scavenger" cells of the body are required. The first is the removal from the circulation of worn out or damaged expendable cells such as red blood cells and leucocytes and the second the disposal of foreign organic material that has penetrated into the tissues. The most satisfactory way to determine the normal distribution of the cells which subserve this double function is to avoid the complications of micro-organismal infection, but to imitate its essentials by the injection of finely particulate material which can be easily recognized histologically. Finely divided carbon (Indian ink) or certain dyes like

trypan blue are the agents most commonly used. These may be injected by various routes so that their ultimate distribution will offer an indication in regard to the spread of foreign material from infections involving different regions of the body. As representative examples we may consider the fate of such substances after injection

(1) into the blood stream,
(2) into the subcutaneous tissue of a limb,
(3) into the peritoneal cavity,
(4) into the subarachnoid space.

If Indian ink is injected intravenously it is very rapidly removed from the blood, and both macroscopic and histological appearances show that it is taken out predominantly in three tissues, in the spleen, the liver and the bone marrow. In all three the carbon particles are found in the modified endothelial cells which line the sinuses or dilated capillaries of these regions. A significant amount of carbon is also removed by the lungs and suprarenals, but much less than in the liver, spleen and bone marrow. Subcutaneous or intradermal injection of suitable dyes shows that much of the inoculum immediately enters lymph capillaries and passes along them to the regional lymph nodes. The local accumulation in the tissues is also gradually drained by the same route. In all tissues dye or carbon particles are predominantly taken up by histiocytes, which may be either fixed or wandering, and which increase in number in response to such local stimuli as a dye injection. Material passing to the lymph nodes is discharged into the cortical sinus and gradually percolates through tortuous spaces lined with active endothelial cells to medullary sinuses and the efferent lymphatic vessels. The lymph node constitutes a well-designed filter and almost all the foreign material arriving from its field of drainage is removed as the lymph passes through the node, being taken up predominantly by the phagocytic endothelial cells which line the rather ill-defined lines of lymph flow within the node.

In the peritoneal cavity there is a rapid taking up of foreign material by the omentum. This contains very numerous histiocytes and small collections of lymphoid

cells and a free lymphatic drainage. Histologically most of the injected material will be found in the histiocytes.

Inoculations into the spaces normally occupied by cerebrospinal fluid result in the carbon or dye being taken up by histiocytic cells, meningocytes, present in the pia arachnoid and particularly numerous in the arachnoid villi by which the cerebrospinal fluid passes out in the dural sinuses.

By experiments of this sort it has been shown that throughout the body there are cells adapted to deal by phagocytosis with foreign material, and the whole system of such cells constitutes the reticulo-endothelial system of Aschoff and Landau. Although isolated cells of this type are present in all regions of connective tissue, they are particularly characteristic of those organs whose structural arrangements make it clear that one of their essential functions is to filter out unwanted material from the blood or lymph streams. These are the liver, spleen, bone marrow and lymph nodes. The first three are blood filters; in each organ we find the normal blood capillaries replaced by wider spaces, sinuses or sinusoids, through which the blood passes slowly and in which foreign material and damaged cells or fragments of cells have many opportunities of coming in contact with the specialized endothelial cells which line the spaces. All three organs have other functions than that of a filter, and liver and bone marrow structure, apart from the presence of sinusoids lined with endothelial cells, is mainly determined by the requirements of these other functions. The structure of the spleen, however, indicates that in all probability its primary function is in some way concerned with maintaining the quality of the circulating blood. It is the only organ of the body in which the blood passes into the actual substance of the organ (in the venous sinuses of the red pulp), and as a natural corollary it lacks any lymphatic system. In the spleen substance the blood filters through a loose reticulum of primitive mesenchymal cells which give rise to and support numerous phagocytic cells of the reticulo-endothelial series. Figure 9 is a diagram after Maximow showing the general structural features

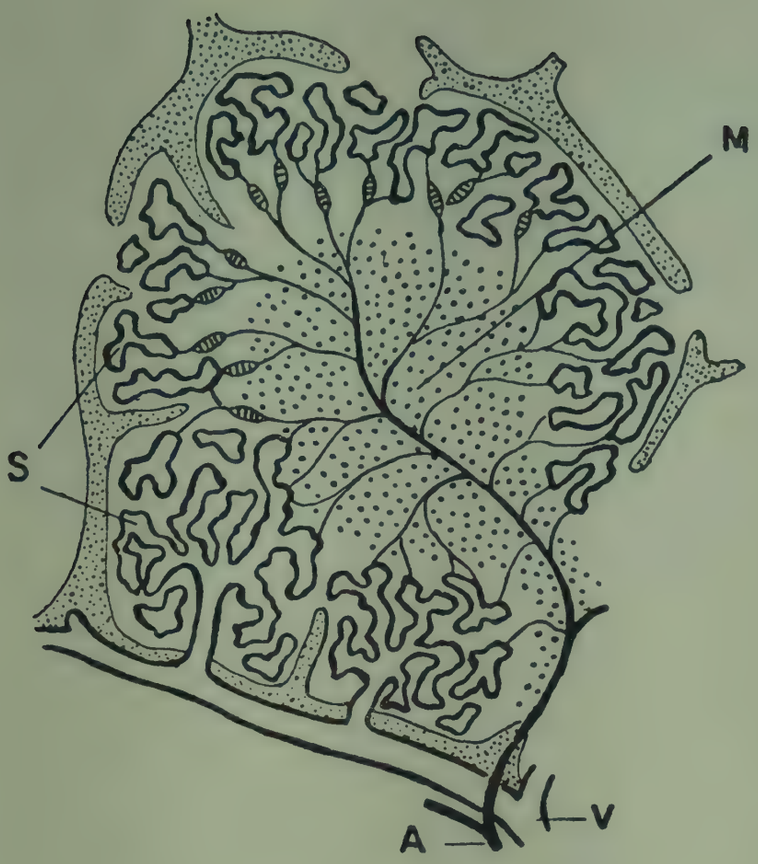


FIG. 9. *Diagram of a splenic lobule, after Maximow* (A, artery; V, vein; S, venous sinuses incompletely lined with phagocytic endothelial cells; M, Malpighian nodule).

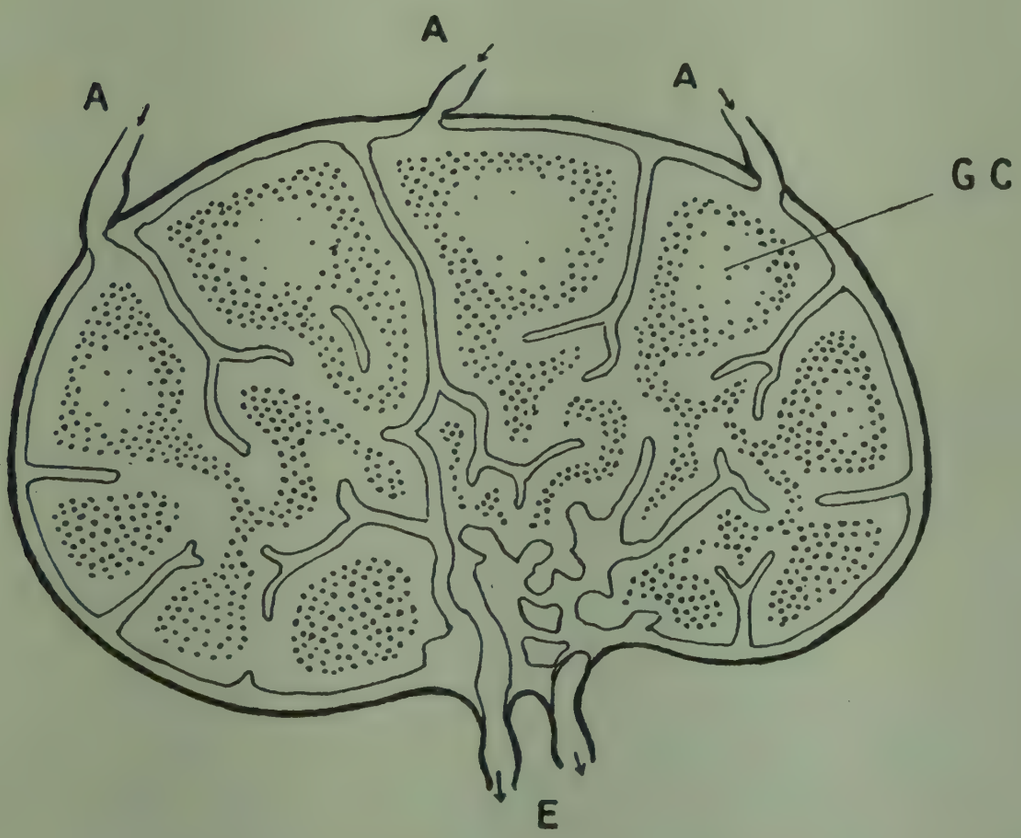


FIG. 10. *Diagram of a lymph node, after Maximow, to illustrate particularly its function as a filter* (A, afferent lymphatics discharging into the cortical sinus; E, efferent lymphatics from the medulla; GC, germinal centre).

of a splenic lobule to make clear its filtering ability. The Malpighian corpuscles which form the whiter granules visible when a spleen is sectioned are composed of lymphoid tissue and their structure, and presumably function, are almost identical with that of the primary nodules of a lymph node. Their only known function is to provide a certain complement of lymphocytes to the blood, but, as will be apparent when we discuss the lymph nodes, they may well have other important activities.

In many respects the lymph nodes can be regarded as miniature spleens, filtering not blood but lymph. The structure is generally similar, as will be seen by a comparison of the diagram of lymph node structure (Figure 10) with Figure 9. The lymph from the periphery enters into the cortical sinus and passes by irregular spaces to the medullary sinuses, from which it is collected by the efferent vessels. According to most authors the sinuses are not defined channels but merely more loosely textured regions of the lymphoid tissue through which the lymph percolates more readily. Denz (1947), however, describes and figures the sinuses as a series of tortuous anastamosing channels penetrating the continuous mass of lymphoid substance. They are lined incompletely with phagocytic cells and may be crossed by strands of reticular cells. The whole arrangement makes a very efficient filter, as has been shown experimentally by Drinker, Field and Ward (1934).

The origin and interrelations of the various cells involved in inflammatory, protective and reparative processes are still a matter for controversy, but apart from matters of detail there seems to be fair uniformity of opinion on basic questions. All the cells are of relatively primitive unspecialized character, and all are of mesenchymal origin. Cells which retain the character and potentialities of embryonic mesenchymal cells persist in most regions of connective tissue, often immediately against capillary endothelial cells forming what are generally termed Rouget cells. In spleen, lymph nodes and bone marrow such primitive cells form a reticulum supported by argyrophile fibrils, within the meshes of which the

other cells of the organs are retained. From these cells the various phagocytic cells of the reticulo-endothelial system may arise in any part of the body. It is probable that in a suitable environment the same primitive mesenchymal cells give rise to lymphocytes (in spleen and lymph nodes) and to granulocytes and erythrocytes (in the bone marrow). The phagocytic mononuclear cells of the reticulo-endothelial system have been given a great variety of names, histiocytes, clasmatocytes, polyblasts, monocytes, macrophages, littoral cells, Kupffer cells or star cells (in the liver), and so on, but from our point of view they may be regarded as essentially a single type of relatively undifferentiated mesenchymal cell, particularly adapted to various scavenging functions.

The cells of the reticulo-endothelial system were defined by experimental dye injections of the sort we have described, but their phagocytic function had been recognized much earlier by Metchnikoff and others in studies on bacterial infection. Such direct evidence of the part played by these cells in dealing with micro-organisms or foreign cells introduced into the body has been obtained from a wide variety of spontaneous infections or experimental procedures. Only a few typical examples may be mentioned. If bacteria are inoculated intravenously into a rabbit they disappear rapidly from the blood. This disappearance is a result of several processes. Many are taken up by polymorphonuclear cells which tend to accumulate in the lung capillaries, while in the liver, spleen and bone marrow they are removed by phagocytic endothelial cells. Both these processes are much more active in immune than in normal animals (Wright 1927 and many others). Perfusion experiments on the removal of bacteria from the perfusion fluid by isolated organs have shown the liver to be the most effective organ, although weight for weight the spleen removes more (Manwaring and Fritchen 1923). The bacteria are removed in the liver by the Kupffer cells. When micro-organisms are taken up by these cells they are usually destroyed, but there are certain types which find the cytoplasm of the endothelial cells suitable for their proliferation. In such circumstances we may find that in-

fection is concentrated in the very cells which normally would be expected to dispose finally of the micro-organisms. The rickettsia of Australian Q fever shows such behaviour in the mouse. After intraperitoneal injection the intracytoplasmic micro-colonies of rickettsiae are found predominantly in cells of the red pulp of the spleen and in the Kupffer cells of the liver (Burnet and Freeman 1937).

For an experimental demonstration of the capacity of lymph nodes to remove bacteria reaching them in the lymph we may refer to the work of Drinker, Field and Ward (1934). They found that if a streptococcal culture was injected into a lymphatic trunk of a dog's hind limb very few organisms reached the blood stream. They were removed predominantly by the cells lining the cortical sinus of the lymph node supplied by the injected lymphatic. Examples of this sort could be multiplied indefinitely, and we may summarize the position in one sentence, that cells of the reticulo-endothelial system are primarily concerned in ridding the body of foreign material or damaged cells and are accumulated at those points where this function can be most usefully fulfilled.

When we have to deal with irregularly occurring emergencies like infectious disease it is obvious that the organism cannot always maintain a full complement of the cells needed to deal with the emergency. The numbers of circulating polymorphonuclear leucocytes vary sharply according to the requirements of the moment, and it is equally necessary that the cells which form the second line of defence should be capable of rapid production when needed. The replenishment of the circulating blood cells is the function of the red bone marrow, but the origin of the mononuclear cells which play a predominant part in all but the most acute local infections is a more difficult problem.

The interrelationships of the various types of cell to be found in the spleen and lymph nodes appear to be still controversial. Maximow held that small and large lymphocytes are cells very little removed from the primitive mesenchymal cell, and that they retain most or all the poten-

tialities of development of the embryonic type. When they emigrate into an inflamed area of tissue, or are allowed to multiply in tissue culture, they take on the character of typical phagocytic histiocytes. Either directly or indirectly they may also give rise to plasma cells and fibroblasts, and with perhaps less certainty they may even, according to Maximow, give rise to haematopoietic cells of erythrocyte and granulocyte series, e.g., when ossification occurs in an old area of inflammation. On the other hand recent authors (Ebert, Sanders and Florey 1940, Medawar 1940) incline to regard at least the small lymphocyte as an end-cell not capable of giving rise to other types. We are probably on safe ground in assuming that both spleen and lymph nodes contain primitive mesenchymal cells (reticulum cells) which under appropriate but as yet unknown stimuli give rise to macrophages, lymphocytes and plasma cells. It seems likely that future developments in histology will be much more concerned with functional qualities than with the labelling and genealogy of cell types. A mesenchymal cell stimulated to take on a certain function may by exercising that function assume the appearance of one or other of the named types. The characteristics of macrophage, plasma cell and lymphocyte are on this view only in a very restricted sense genetically determined. It may be as Fagraeus' (1948b) work implies, that when a mesenchymal cell is actively producing new protein which will eventually appear as plasma globulin it takes on the staining qualities of the plasma cell. This is not quite the same as saying that plasma cells are cells whose function is to produce globulin.

In the next section recent work on the function of the lymph nodes as centres of antibody production will be reviewed. Here it is desirable to discuss two types of histological appearance in spleen and lymph nodes to which importance in regard to antibody production has been ascribed, the development of germinal centres and the appearance of increased numbers of plasma cells.

The work of Hellman and his collaborators (Hellman and White 1930) in Sweden has been directed toward elucidating the significance of the germinal centres of

Flemming which are found in the lymphoid tissue (Malpighian nodules) of the spleen and in the follicles of the lymph nodes. (See Figures 9 and 10.) In sections the germinal centres appear as pale areas surrounded by a dense ring of close-packed small lymphocytes. Their pallor is due to the presence of cells with larger amounts of cytoplasm and with less deeply stained nuclei. Most of these cells may be called large or medium lymphocytes, but there is no sharp distinction to be made between any of these groups. It is usual to find also damaged cells and fragmented nuclei largely ingested by histiocytic cells in a proportion of the germinal centres. In both spleen and lymph nodes germinal centres are characteristically variable in number and are obviously transient structures. According to Maximow they arise always in relation to a small arteriole which in the resting state is surrounded by a few primitive pale-staining nuclei in a syncytial protoplasm. These are portion of the reticulum, and from them develop medium sized lymphocytes and the other cells of the germinal centre. The disappearance of germinal centres presumably results from maturation of the cells to small lymphocytes so that the whole follicle takes on again the solid appearance.

The significance of the germinal centres has been a matter of controversy, one school regarding them as centres of production for the blood lymphocytes, while Hellman and his followers refer to them as reaction centres rather than germinal centres, holding that their appearance is a response to any toxic or foreign material which may reach the spleen or lymph node. Ehrich (1946) considers that they may also be in part regions where lymphocytes are being returned from the blood to the lymph nodes. The spleen and lymph nodes of the foetus or very young animal do not contain germinal centres. Glimstedt (1936) found that young guinea pigs removed by Caesarean section and reared in a sterile environment for one or two months gained weight normally and appeared to be in good health. Their lymphoid tissue was diminished in amount in comparison with control animals in a normal bacterially contaminated environment, and there was an

almost complete absence of germinal centres. This speaks strongly for the view that parenteral entry of foreign material (usually bacteria) is the immediate stimulus to the appearance of germinal centres. Negative evidence in the same direction is given by Sjovall's (1936) failure to influence the number of germinal centres by repeated bleedings, which caused a great compensatory hypertrophy of the bone marrow. If supply of lymphocytes to the blood had been the primary function of the germinal centres they should have been expected to respond like the bone marrow to the stimulus of repeated haemorrhage.

Many authors have shown that following the administration of killed bacteria or other foreign antigenic material to rabbits there is a sharp increase in the number of germinal centres along with other associated changes in spleen and other lymphoid tissues (Hellman and White 1930, Ehrich 1929). In the lymph nodes draining a region of subcutaneous infection with low-grade staphylococci Ehrich (1929) found first oedema and enlargement of the node with the appearance of solid pseudo-secondary nodules of lymphocytic proliferation. Germinal centres did not appear until seven to fourteen days after inoculation. After intravenous inoculation with killed staphylococci the spleen showed increase in the size of Malpighian bodies and an enormous increase in the number of Flemming's secondary nodules (germinal centres). Osterlind (1938) injected diphtheria toxoid subcutaneously in the hind limb of rabbits. The regional lymph node showed great enlargement, reaching its maximal size on the third day. The number of germinal centres did not increase, but their average size was larger. In rabbits with a basic immunity to diphtheria toxin the response is different. There is the same early swelling and oedema with some general mitotic activity and phagocytosis of damaged cells. Then about the fifth to the eighth day there is an appearance of new germinal centres which enlarge rapidly, pushing the small lymphocytes of the follicle to the periphery. This period over which new germinal centres were appearing and rapidly enlarging coincided with that of the rapid secondary production of circulating antitoxin. As a result of

this work done in Hellman's laboratory, Osterlind considers that the reaction centres are "specially designed antibody producers".

The activity of the germinal centres in lymph nodes and spleen is not the only distant cellular response to local infection or the injection of various foreign materials. Ehrich (1929), confirming earlier work by Oeller (1923), found that after inoculation of dead staphylococci intravenously in rabbits accumulation of mesenchymal cells, both lymphocytic and histiocytic in type, rapidly appeared in liver, lung and spleen, particularly in the perivascular tissue associated with the smaller blood vessels. Thompson (1922) found an increase in the number of phagocytic cells in the spleen and liver after tuberculin injections in rabbits, with a tendency toward the formation of giant cells. Rous and Beard (1934), in the course of a detailed study of the isolated Kupffer cells of the rabbit and dog, made a number of incidental observations on the conditions under which large amounts of cells could be set free from the liver by perfusion and massage. Although very few could be obtained from a normal liver, three to five days after the last of three injections of ferric oxide gum acacia suspension enormous numbers could be obtained. This resulted both from increase in the number of cells and loosening of their attachment to the wall of the liver sinusoids. Intravenous injection of Indian ink had the same effect.

Similar increase in the number and the size of Kupffer cells and in their tendency to be liberated into the circulation were noted by Epstein (1929) after intravenous administration of bacterial antigens in the rabbit. He also described a characteristic change toward more basophilic staining giving the cells very much the appearance of plasma cells.

The suggestion that the plasma cell may be the most important producer of antibody and serum globulin is almost wholly due to Scandinavian workers and most of our discussion of this topic is based on Fagraeus' recent monograph (1948b). Although Huebschmann (1913) from a study of plasma cells in the spleen suggested that

plasma cells are most abundantly present where specific antibody is being most actively produced, recent interest in the subject is derived from the association between hyperglobulinaemia and undue accumulation of plasma cells either as myelomata or in other conditions (Bing and Plum 1937, Undritz 1938). In 1943 Bjornboe and Gormsen described increased plasma cell accumulation in various organs of hyperimmunized rabbits and in a subsequent paper (Bjornboe, Gormsen and Lindquist 1947) showed that tissue extracts from such animals showed antibody titres more or less closely proportional to the plasma cell content of the tissues. The co-existence of large numbers of plasma cells and high antibody content in the adipose tissue of the renal pelvis was probably the most impressive of their findings. Fagraeus (1948b) studied particularly the changes of the cellular content of the splenic red pulp in relation to the various stages of the antibody response to a secondary antigenic stimulus. She also determined the production of antibody in tissue cultures prepared from red and white spleen pulp from immunized animals and correlated the results with the cytological appearance of the cultures. Her conclusions were that the antibodies with which she was concerned were produced by cells in the process of transition from reticulum cells to plasma cells.

ANTIBODY PRODUCTION IN LYMPH NODES

The lymphoid tissue has long been thought to be concerned in antibody production (Murphy and Sturm 1925), but experimental proof of the origin of antibodies in lymph nodes is of comparatively recent origin. McMaster and Hudack (1935) showed that after the inoculation of two different antigens into the two ears of mice, the corresponding agglutinin appeared first in the cervical lymph node of the same side, and the agglutinins appeared earlier in the lymph nodes than in the serum. Later McMaster and Kidd (1937) showed that when rabbits were inoculated on the ear with vaccinia virus, antibody developed in the homolateral cervical lymph node in four

days and was then in greater concentration than in the serum.

Burnet and Lush (1948b) found that in mice infected intranasally with a strain of influenza virus of low virulence very little virus was detected in the mediastinal lymph node at any time, but from the fifth day onwards neutralizing antibody could be detected. From the fifth to seventh days there was more antibody in the node than in the serum, and it was suggested that practically all the antibody produced came from this source. No appreciable amounts of antibody could be detected in the lung, so that the evidence for its production in the lymph node is almost conclusive. Other experiments by the same workers (described in detail in the first edition of this monograph) showed that after scarification of the rabbit cornea with herpes simplex virus, specific antibody appeared sooner in the local lymph node than in the serum. Experiments in which rabbits were inoculated in the pad of the foot with a culture of dysentery bacilli lysed by phage C16 showed that both antiphage antibody and agglutinin to the homologous strain of the dysentery bacillus were present in highest titre in the corresponding popliteal lymph node on the fifth and sixth days.

In a series of papers Ehrich, Harris and their colleagues described the cellular and antibody content of the efferent and afferent lymph from the popliteal lymph node, as well as the serum antibody level, after the inoculation of particulate antigens (typhoid bacilli and sheep erythrocytes) into the hind foot of the rabbit. They also studied the cellular response at the site of inoculation and in the regional lymph node at various periods after the inoculation of the antigen. They found firstly (Ehrich and Harris 1942) that the concentration of antibody was always higher in the efferent lymph than in the afferent lymph and that this antibody rise was preceded and accompanied by a rise in the output of lymphocytes in the efferent lymph. The cellular response in the lymph node consisted of greatly increased lymphocytopoiesis, and this preceded the rise in the output of lymphocytes and the level of antibody in the afferent lymph. Next (Harris, Grimm,

Mertens and Ehrich 1945) they showed that if the lymphocytes of the efferent lymph were separated from the lymph plasma, the antibody titre of the cell extract was considerably higher than that of the lymph plasma, the difference being greatest at the time of the greatest rate of increase of the antibody titre of the whole lymph. *In vitro* and *in vivo* experiments showed that lymphocytes did not take up antibody from lymph, and the authors concluded that the lymphocytes were instrumental in the formation of antibodies. Subsequent investigations were concerned with the possible role of macrophages in antibody formation. When dysentery bacilli in paraffin oil were inoculated in the foot pad of the rabbit (Ehrich, Harris and Mertens 1946) the tissue at the site of inoculation contained many granulocytes and macrophages but negligible amounts of antibody. Macrophages in the peritoneal exudate, obtained after the intraperitoneal inoculation of dysentery and typhoid bacilli and various irritants, were also devoid of antibody. However, by investigating the concentration of antigen, (sheep's erythrocytes or dysentery bacilli), or soluble products of the antigen, as well as antibody, in extracts of the injected tissue and the regional lymph node, and in the efferent lymph, Harris and Ehrich (1946) showed that the concentration of the soluble material fell off slowly in the inoculated tissue, but declined quickly in extracts of the lymph node and in the lymph itself, its disappearance being succeeded by the appearance of antibody. They suggested that the role of the macrophage might be to break down the foreign cells and release immunologically active soluble substance which then passed to the lymph node and was there responsible for the production of antibody by the lymphocyte.

All these investigations show that for a wide variety of particulate antigens, red cells, bacteria, viruses and bacteriophages, peripheral inoculation results in early formation of the appropriate antibody in the lymph node draining the site of injection or of virus proliferation. All these antigens are of the type which produces an active primary response. When staphylococcal toxoid was

similarly studied as the most convenient representative of those antigens which show a sharp difference between primary and secondary antibody response, no such clear evidence of antibody production in the local lymph node could be detected. Since no work on this theme has subsequently been published we include the account of the relevant experiments from the first edition of this monograph.

Attempts to Detect Significant Production of Staphylococcal Antitoxin in the Popliteal Lymph Node

Since the primary antitoxic response is of such small extent, it seemed that the only practicable method of detecting antibody production in lymph nodes would be to investigate the secondary response. In the first experiment four rabbits were given a single dose, 0.5 c.c., of staphylococcal toxoid intravenously. Nineteen days later they were given a dose of 1 c.c. of toxoid subcutaneously in the right foot. A sharp rise in antitoxic titre of the serum commenced on the third day and the rabbits were killed on the fifth day, when one could be reasonably certain that the secondary production of antitoxin was at its height. As in the other experiments, the lymph nodes on each side were removed, cleared of fat and weighed. A 5 per cent emulsion was made in saline, and after centrifugation the supernatant fluid was titrated for its antitoxin

Table 1

ANTITOXIN IN LYMPH NODES OF RABBITS RECEIVING A SECOND INJECTION OF STAPHYLOCOCCAL TOXOID IN THE RIGHT FOOT AFTER A PRELIMINARY INTRAVENOUS INJECTION OF TOXOID

Rabbit	*Primary serum titre*	*Serum titre when killed*	*Right lymph node*		*Left lymph node*	
			Weight (gm.)	*Antitoxin*	*Weight (gm.)*	*Antitoxin*
31	0·05	5·4	0·394	0·8	0·090	0·4
32	0·16	9·8	0·345	0·9	0·102	0·55
33	0·13	5·9	0·606	1·3	0·196	< 0·3
34	0·98	18·5	0·544	2·0	0·194	0·8

All antitoxin titres are expressed in international units.

content. This is expressed in the tables as units of antitoxin per gram of tissue.

The results are quite clear; there is a considerable enlargement of the lymph node on the inoculated side, but there is only a barely significant difference in the antitoxic titres. On the average there is about twice as much in the node on the inoculated side as in the other. The whole difference could be readily accounted for by the reasonable assumption that a higher content of serum globulin is present in the enlarged and inflamed node.

Various experiments were carried out to determine whether any more significant results could be obtained. A series of rabbits was killed at four days after the subcutaneous injection when the serum titre was still low. In this group there was no antitoxin in the left node, and only the smallest detectable trace in the right. With a single dose of toxoid subcutaneously there was no trace of antitoxin in the corresponding lymph node four days later. Another series received two subcutaneous inoculations of staphylococcal toxoid; in two rabbits both inoculations were into the same foot, in two others the opposite foot was used for the second inoculation. There was again a just significant difference between the two lymph nodes.

In view of these results with a staphylococcal toxin we are left very doubtful as to the significance to be placed on the inflammatory-proliferative changes in the lymph nodes which were observed by Oesterlind in rabbits treated with diphtheria toxoid. It seems probable that the cellular proliferation observed was not to be directly related to the production of diphtheria antitoxin.

The rather striking difference between the two types of antigen calls for some explanation. Probably the simplest way to co-ordinate the results is to assume that the local lymph nodes are especially fitted to deal with particulate antigens such as bacteria and viruses, but that bacterial toxoid, perhaps because of its much greater diffusibility, is not significantly taken up by the lymph nodes. Either by diffusion into the local blood capillaries or after passage through the lymph nodes, it passes almost entirely into the

blood and antitoxin is produced elsewhere. If one can generalize from Buttle's (1934) experiments on the secondary antitoxic response to diphtheria toxoid in rabbits, antitoxin may be produced at a rapid rate even when spleen, liver and skin have been eliminated from the circulation. In such a preparation, cells in the bone marrow are presumably responsible for most of the antitoxin increase observed.

The suggestion that more than one type of cell is concerned in antibody production is supported from a different direction by a recent paper by Carlinfanti (1948). He compared the efficiency with which individual animals produced antibody against different types of antigen and found that there was a positive correlation in the extent of antibody production when antigens of related types were considered. Thus an animal which produced high titre antibody against somatic bacterial antigens also produced high titre flagellar antibodies, and one which produced high titre diphtheria antitoxin also produced tetanus antitoxin in large amount. There was, however, little or no positive correlation between capacities to produce antibacterial antibodies on the one hand and antitoxins on the other, nor were these correlated with titres of natural antibodies (iso- and hetero-haemagglutinins or haemolysins).

The evidence suggests that different cell systems or different cellular processes are involved in the production of different types of antibody and that the efficiency of these systems is to some extent at least genetically controlled.

THE ROLE OF MACROPHAGE, LYMPHOCYTE AND PLASMA CELL IN ANTIBODY PRODUCTION

The preceding account makes it clear that with particulate antigens inoculated at a peripheral site the major production of circulating antibody takes place in the regional lymph node. The question immediately arises as to which of the various cells present in the lymph node are responsible for this production. Macrophages, lymphocytes and plasma cells are all present in the normal lymph

node, an active increase in each type is evident in nodes reacting to infection or the inoculation of antigenic material and each has been considered as being predominantly responsible for antibody production by one author or another.

There is no controversy over the fact that visible antigens, bacteria or artificial dye-antigens are taken up primarily by the macrophages lining the lymph sinuses and that no signs of the antigen can be seen either in lymphocytes or plasma cells (Fagraeus 1948). According to the latter author plasma cells appear in large numbers in the medullary cords in close association with the antigen-containing macrophages. They are absent in the germinal centres.

Ehrich (1946) considers that the lymphocytes are at least the major producers of antibody both in the lymph nodes and the spleen.* This opinion is largely based on the experiments by Ehrich and his collaborators described in the preceding section but it is also strongly influenced by the work of White, Dougherty and their colleagues. These investigators have provided evidence that lymphocytes are concerned with the carriage and liberation into the blood plasma of normal and immune γ globulin and β globulin. They approached the problem as physiologists interested in endocrine reactions. The observation of the inverse relationship between the degree of adrenal cortical secretion and thymic size led to the study of the response of other lymphoid structures to adrenal cortical hormones. It was found that lymphoid tissue underwent involution as a result of augmented pituitary-adrenal cortical secretion, and that at the time of maximal involution there was a profound lymphopenia (Dougherty and White 1944). Histological studies suggested that both the decrease in lymphoid tissue and the blood lymphopenia were the result of the "dissolution" of lymphocytes, a liberation of lymphocyte protoplasm which occurred in the lymphoid

* Ehrich (1949) now considers that antibody production in lymph nodes is a function of plasma cells and ascribes the earlier findings of antibody in the "lymphocyte" extracts as being due to the presence of plasma cells in the tissues and fluids concerned.

organs as a consequence of augmented pituitary-adrenal cortical secretion (Dougherty and White 1945). The observations of Yoffey (1933, 1936), Sanders, Florey and Barnes (1940) and others have shown that the blood lymphocytes are replaced at least two or three times daily, and the fate of the lymphocytes which disappear so rapidly from the circulatory blood has been studied by many investigators. Many are lost by passage through the intestinal mucosa to the lumen of the gut (Bunting and Huston 1931). Dougherty and White consider that the other important mode of destruction of the lymphocytes is their dissolution in the germinal centres of the spleen and lymph nodes. Dissolution includes all changes from the shedding of cytoplasm to necrosis, and they found that while dissolution occurred in normal animals it is greatly enhanced by the injection of adrenotrophic or adrenal cortical hormones. They next investigated the relationship between the proteins of the lymphocyte cytoplasm and the serum proteins (White and Dougherty 1945) and found firstly that the injection of adrenal cortical hormones caused a considerable elevation in the β and γ globulin concentrations in the serum, and secondly that lymphocyte extracts contain components which are electrophoretically identical with β and γ globulin. The demonstration of γ globulin in lymphocytes led to investigations of the antibody content of lymphocytes of immunized animals (Dougherty, Chase and White 1944), and it was shown that lymphocyte extracts from immunized animals contained larger amounts of antibody than did the serum, while those from normal animals contained none. This was true both for antibodies to particulate antigens, and for staphylococcal antitoxin developed in mice after a series of subcutaneous inoculations of staphylococcal toxin (Dougherty, White and Chase 1945).

It was further shown that release of the antibody globulin in lymphocytes, like the normal γ globulin, was controlled by the adrenal cortical hormones. Antibody was found in lymphocyte extracts of immunized animals after it had disappeared from the serum, and the administration of adrenal cortical hormones to such animals (Dougherty,

Chase and White 1945) caused the appearance of antibody in the serum. By using adrenal cortical steroids and the pituitary adrenotrophic hormone, and large and small doses of X-rays in normal and adrenalectomized immunized mice (White and Dougherty 1945) it was shown that the action of the adrenal cortex in producing increases in serum protein and antibody was based on the dissolution of lymphocytes by the steroid hormones of the adrenal gland.

We are inclined to doubt whether this work has the full significance ascribed to it by the authors. It is hardly admissible to ascribe all the properties shown by lymph node extracts to the lymphocytes, an objection which also applied to Ehrich's studies on the cellular components of efferent lymph. The anamnestic appearance of antibody in previously immunized animals after appropriate hormonal treatment was of no great magnitude and might be due to processes unassociated with lymphocyte destruction.

The evidence in favour of the plasma cell rather than the lymphocyte being the main source of antibody production in lymph nodes (and spleen) is summarized by Fagraeus as follows: (1) The injection of an active antigen, ovalbumin, in rabbits produces a sharp increase in plasma cells in the spleen; physically similar, non-antigenic substances like gelatine, peptone and dextran produced no such effect. (2) In the course of a secondary antigenic response cytological studies of the spleen showed first a proliferation of large reticulum cells with an increased cytoplasmic basophilia (transitional cells) followed by their development in the direction of typical plasma cells. At the time of most intense new formation of antibodies immature plasma cells predominated. (3) In tissue culture experiments with spleen fragments from immunized rabbits the red pulp produced more antibody than white pulp; the capacity of the red pulp to produce antibody was directly related to its content of plasma cells, particularly immature plasma cells.

To these should be added the finding by Bjornboe and collaborators that in hyperimmunized animals the antibody

content of tissues is correlated with the numbers of plasma cells visible in histological sections.

We may summarize the present position with some diffidence as indicating

(1) That antibody production against particulate antigens introduced into local tissues is predominantly a function of the lymph nodes draining the region.

(2) That the spleen plays an essentially similar part when the antigenic material circulates in the blood.

(3) That antibody produced in the lymph node passes to the circulation largely in the cytoplasm of the cells (lymphocytes and plasma cells) in the efferent lymph stream.

(4) That liberation of antibody from these cells may in part or wholly be due to cytoplasmic dissolution induced or controlled by hormones of the adrenal cortex.

These conclusions apply only to serum antibodies developed in response to the inoculation of particulate antigens. There is no certain evidence as to where the antibodies concerned with sensitization reactions or the response to tissue grafts are produced and virtually no evidence whatever as to how they are carried to the general tissues of the body.

LOCAL PRODUCTION OF ANTIBODY

We have already referred to the experiments of Ehrich *et al.* (1946) in which they failed to find evidence of local antibody production to a dysentery bacillus vaccine despite the presence of large numbers of macrophages at the site. This negative finding is not necessarily of general application. Evidence of an accumulation of homologous antibody at the site of infection or inoculation with killed bacteria is given by Cannon and Sullivan (1932) for the skin of the rabbit and by Walsh, Sullivan and Cannon (1932) for the nasal mucosa. Their evidence is suggestive, but does not appear to have been adequately controlled to exclude the possibility of accumulation of antibodies produced elsewhere, at any site of inflammatory change.

That such accumulation can occur is suggested by the work of Menkin (1930, 1938) and of Fox (1936).

In our own experiments each rabbit was injected with four different antigens into areas of skin previously treated by injection of a little sterile quartz powder emulsion. After either one or two injections of the antigens the rabbits were killed and the antibody content of serum and of extracts of each skin area was estimated. The antigens used were staphylococcal and diphtheria toxoids, phage C16 and a bacterial vaccine. The staphylococcal toxoid lesions were mildly inflamed, presumably on account of incomplete detoxication of the toxin, and in the extracts from these skin areas all antibodies were in higher concentration than in the others. There was no evidence of any local production of antibody in the skin.

More definite evidence of local production of antibody is to be found in Oerskov and Andersen's (1938) study of the content of vaccine virus in rabbit skin lesions at various periods after inoculation. Table 2 modified from their paper shows the results of titrating ground-up lesion emulsions from rabbits inoculated with a series of falling dilutions of a stock virus.

The only possible interpretation of these results is that with a large virus inoculum local formation of antibody takes place rapidly, and its presence masks the activity of the virus which is undoubtedly also present. Direct evidence was also obtained that extract from a four-day

Table 2

INFECTIVITY OF VACCINIAL SKIN LESIONS IN RABBITS AT VARYING PERIODS AFTER INOCULATION (OERSKOV AND ANDERSEN)

	Dilutions inoculated in original rabbits			
	10	10^2	10^3	10^4
One-day lesions ..	640+	640+	640+	40
Two-day lesions ..	±	±	320	640+
Three-day lesions .	0	0	640 aty.	640+

The figures show the dilution to which the lesion emulsion was infective on intradermal inoculation in normal rabbits.

lesion contained demonstrable virus-neutralizing antibody.

Rather similar results were obtained by Stone and Burnet (1946) in their work on the production of vaccinia haemagglutinin in the rabbit skin. The rapid disappearance of the haemagglutinin in the lesions was ascribed to precocious local production of antihaemagglutinin.

Recent investigations by Morgan (1947a) have provided evidence of the local formation of antibodies in the central nervous system. Monkeys which recover after paralytic infection with poliomyelitis virus inoculated by the intracerebral or intranasal route are solidly immune to reinfection with the homologous strain of virus, even though the serum antibody titre may be low, whereas monkeys vaccinated with living virus inoculated intramuscularly show immunity to intracerebral challenge only if the serum antibody titre is very high. By testing serum, lymph node extract, spinal fluid, and grey and white matter from the central nervous system for neutralizing antibody, Morgan found that the titre of grey matter from the susceptible anterior horn region had a very high antibody content, which remained elevated for months after the serum antibody had disappeared. She considered that this antibody was probably formed locally in the infected regions of the brain and spinal cord where both macrophages (including microglial phagocytes) and lymphocytes accumulate during the gliosis and perivascular inflammatory reaction in acute poliomyelitis.

The special problems concerned with the appearance of allergic reactivity as a result of certain types of localized infection are more conveniently discussed in a later section.

The only general conclusion that seems to be justified is that antibody production is a function of natural or temporary accumulations of mesenchymal cells which include macrophages, lymphocytes and possibly other functional types. It seems certain that the nature of the cells involved depends on the type of antigen and the type of antibody being produced. In a later section we shall return to the question of the respective parts played by macrophage, plasma cell and lymphocyte when the general question of the nature of antibody is discussed.

CHAPTER VI

THE EVIDENCE THAT ANTIBODY PRODUCTION MAY CONTINUE IN THE ABSENCE OF ANTIGEN

We have already noted that following certain infections specific antibody may continue to be present in the serum for many years. Evidence has also been given that antibody production continues at a diminishing rate for some time after the maximal serum concentration has been reached. From the theoretical aspect it is important to determine if possible whether antibody production continues only so long as antigen remains present in the antibody-producing cells. Largely on account of Pauling's (1940) adoption of the view that antibody is synthesized in direct contact with the determinant groups of the antigen, this is at present the most generally favoured conception. In our view, however, the facts suggest more strongly that once the antigenic stimulus has been applied the cells may continue to produce antibody long after the antigen has been completely disintegrated.

It is certain that microscopically visible antigenic particles, red cells and bacteria are rapidly disintegrated in the reticulo-endothelial cells into which they are taken. From what is known of intracellular enzymes one would presume that, simultaneously with the disappearance of visible structure, the structure of the antigen molecules composing it would also be disintegrated. It is not easy, however, to provide direct evidence against the view that the essential antigenic determinants are retained as a kind of template which impresses the specific pattern on each antibody globulin molecule as it is synthesized. The most direct experimental attack would be to immunize with an artificial antigen carrying as determinant group an organic arsenical configuration, e.g., arsanilic acid, and to follow its disappearance from the spleen, liver and bone marrow. Unfortunately such artificial antigens do not

provoke such large and easily measured antibody responses as are obtained from natural particulate antigens like bacteria, and it might not be easy to obtain evidence of, e.g., regeneration of antibody after bleeding some months after immunization.

A more important difficulty would be the tendency for arsenic to persist in the cells after the original compound has been disintegrated. Haurowitz and Kraus (1936) give some data on this distribution of antigens containing arsenic or iodine after intravenous injection. As would be expected, they accumulate rapidly in liver, spleen and bone marrow. The subsequent decrease, as judged by arsenic or iodine estimations of these tissues, is much more rapid with the iodoglobulin than with an arsenical protein. The arsenic was found mostly combined with insoluble structural protein in the cells, and was probably not in its original organic form. The iodine broken off from the iodoglobulin is readily excreted and the data from the experiments with this substance which showed 95 per cent or more of the iodine disappearing within six days are probably more directly applicable to antigens in general. In this case, however, no data are given as to whether the iodine atoms are included in any essential antigenic determinants of the protein used, or whether a persisting antibody response followed immunization. The findings therefore offer only indirect support for the view that antigen disappears from the cell long before the cell loses its capacity to produce antibody.

Another approach of essentially similar character is to use an antigen "labelled" with a radioactive isotope. The only experiments so far reported along these lines are those of Libby and Madison (1947) who used as antigen tobacco mosaic virus protein containing radioactive phosphorus. The experiments were designed to follow the disappearance of the antigen in relation to antibody response. Basically immunized mice were given an intravenous injection of the labelled antigen. The antibody response actually studied was therefore the brief period of a secondary-type antibody rise. The decay of radioactivity in liver and spleen indicated an initial "half-life" of the anti-

gen of two days followed by a subsequent phase with a half-life of nine days. The author's interpretation that the first represents the rapid disintegration of the antigen and the second the slower disappearance of P-containing fragments from the antigen which had been utilized metabolically by the body cells, seems a highly probable one. In the theoretical analysis by Salley and Libby (1947) Pauling's theory is used as a basis and the conclusions are reached (1) that antibody production ceases as soon as the antigen is destroyed and (2) that to a rough approximation for each four molecules of antigen which are decomposed, 150 molecules of antibody are produced and one molecule of antibody decomposed. In our opinion this interpretation probably only approaches validity for the sharp rise and fall of the secondary response. The antibody curve published by Libby and Madison shows no fall over the last six days of their experimental period of 31 days and general experience would indicate that this low level would then have fallen only very gradually over many weeks if the experiments had been continued. In our opinion this work is of importance in demonstrating the rapidity with which an antigen is disintegrated in the body and does not indicate that antibody production ceases with its disappearance.

There is no lack of other indirect evidence to show that the antigenicity of the antigen is rapidly lost once it is taken up by the reticulo-endothelial cells. Topley (1930) transferred finely ground splenic tissue from rabbits injected intravenously with killed *S. typhi* 24 hours previously, to the peritoneal cavity of normal rabbits. A few days later the recipients showed small amounts of agglutinin which in some of the experiments at least must have been produced by the donor's cells (or perhaps cell components) while they were in the recipient's body. Evidence that the antibody was not produced by the recipient's cells taking up antigen introduced with the splenic tissue emulsion is provided by the fact that the animals did not give a secondary response to a subsequent injection of a small dose of the same antigen. The results indicate that within 24 hours a bacterial vaccine has been

rendered non-antigenic by being taken up in the splenic histiocytes. Price (cited by Northrop, Kunitz, and Herriot 1948) made similar experiments using crystalline yeast hexokinase as antigen. He found that splenic extracts made after the lapse of 24 hours from intravenous injection of the enzyme contained no detectable hexokinase. Such extracts inoculated into fresh rabbits produced a significant production of antibody but did not induce a state of secondary reactivity to the original antigen. Northrop considered that these experiments were best interpreted by assuming that the hexokinase was rapidly broken down to the effective antigen, losing its enzymic activity in the process, and that this effective antigen was responsible for provoking antibody production either in the originally inoculated animal or in the recipient of the splenic extract. While it is by no means unlikely that some breakdown products of a complex enzyme, lacking enzyme activity, can provoke antibody capable of inactivating the enzyme, Price's experiments give no indication of any prolonged retention of the "effective" antigen. It, too, is probably destroyed rapidly.

The only example we have been able to find in which there appears to be real evidence of persistence of antigen for a considerable period is in regard to immunization in human beings and mice with purified pneumococcal polysaccharides. Schumann and Casper (1927) were the first to show that mice could be specifically immunized by soluble derivatives of pneumococci and that to be effective the amount administered as immunizing dose must not exceed a certain very small amount. This was confirmed by Wadsworth and Brown (1931), Felton (1934) and others. Felton described preparations effective only in dilutions between 10^{-5} and 10^{-8}. Heidelberger *et al.* (1946) in studying quantitatively the antibody response to purified pneumococcal polysaccharide in human subjects make two important points: (1) The very slow fall in the amount of precipitable antibody over a period of two years. In most instances the drop was to about one third of the peak titre reached a few weeks after immunization. (2)

The failure of secondary response to a reinjection of the same antigen even two years after the first injection.

Possibly related to these phenomena is the finding of Dubos and MacLeod (1937) that pneumococci are rapidly rendered non-antigenic (in respect to type-specific precipitating antibody in rabbits) by treatment with leucocytic extracts or purified ribonuclease.

The possibility is raised by Heidelberger that the specific polysaccharide has these qualities because it is retained within the antibody-producing cells and in so doing blocks any opportunity for subsequently injected antigen to influence the antibody-producing mechanism.

These phenomena appear to be restricted to one unusual type of antigen and though their fuller investigation will probably throw new light on the intracellular processes involved, it is legitimate to state that for most types of antigen their molecules are disintegrated in the macrophage.

The lifetime persistence of immunity against certain acute virus infections, particularly measles and yellow fever, is firmly established by epidemiological evidence. The classical example is the measles experience of the Faroe Islands in the first half of the nineteenth century (Panum 1847). Successive epidemics reached the Islands in 1781, 1846, and 1875, each epidemic affecting, with hardly an exception, all those who had not been infected previously and leaving untouched those who had. The use of adult serum in the prophylaxis of measles makes it certain that specific antibody persists for some years at least. Until a method of titration is available however, it must remain only the most probable assumption that lifelong immunity to measles is associated with lifelong production of specific circulating antibody. In the case of yellow fever, the evidence for persisting immunity is equally strong, and in addition, there is direct evidence that a single infection may induce the formation of antibody which can be detected in the serum 75 years later (Sawyer, 1931).

Similar evidence that a single attack of Rift Valley fever can result in the production of neutralizing anti-

bodies for at least twelve years has recently been provided by Sabin and Blumberg (1947).

It is quite impossible to think of reticulo-endothelial cells maintaining individual existences for 75 years, or of fragments of virus being retained as antigenic pattern for so long. A more rational hypothesis would be to consider that the virus may remain living and slowly multiplying for years without symptoms in the immune animal and provide a constant or intermittent stimulus for further antibody production. Such persistence of infection in the immune animal is known to occur in such conditions as trypanosomiasis or piroplasmosis, in many rickettsial infections, and in infections with the virus of herpes simplex. In all these, however, there is clear epidemiological evidence of the persistence of infection. An infected individual is a carrier who, on entering a non-immune population, will sooner or later transmit infection to the non-immunes. This certainly does not occur in measles or yellow fever, and there is no reasonable escape from accepting the long-lasting immunity as something induced by the infection, but maintained by the body after all the virus has been destroyed.

CHAPTER VII

IMMUNOLOGICAL BEHAVIOUR OF YOUNG ANIMALS

ANY attempt to understand the physiology of the immune reactions must take into account the interesting immunological phenomena observed with the change from the embryo in a sheltered, uncontaminated environment to the free-living young exposed to infection by many types of micro-organism. In general it is found (1) that there is a mechanism by which maternal antibody globulin is supplied passively to the offspring, (2) that the young animal is much less reactive to toxic and infective agents than the adult, and (3) that for a short time the young are almost incapable of producing antibody.

TRANSFER OF MATERNAL ANTIBODY GLOBULIN

In birds the yolk contains antibody that was present in the fowl's blood, at a titre ten to a hundredfold lower than the serum titres of the hens. This is taken up by the embryo during incubation and persists in the serum at peak level for two or three weeks after hatching. This has been shown for diphtheria antitoxin (Jukes, Frazer and Orr 1934), for tetanus antitoxin by Ramon (1928), and for neutralizing antibody to Newcastle disease virus by Brandly, Moses and Jungherr (1946). Schmidt, Oerskov and Steenberg (1936) found that chicks hatched from eggs laid by fowl plague immune hens were immune for a few weeks after hatching. Andrews (1939) noted that Rous sarcoma antibodies were transmitted in similar fashion.

In mammals, transfer of maternal antibody may take place either through the placenta, via the colostrum, or via the milk. Among the ruminants the young are born without antibody, and according to Jameson, Alvarez-Tostado and Sortor (1942), Polson (1943) and Hansen

and Phillips (1947), without γ globulin in their serum. In cattle, Smith (1930), Minett (1937) and Miller and Heishman (1943), goats, Famulener (1912), and sheep, Mason, Dalling and Gordon (1930) the colostrum often contains the maternal antibodies in higher concentration than they are present in serum taken at the same time. Antibody could sometimes be detected in the milk, but its concentration was always very low. Smith and his collaborators (Smith 1946 a and b, Smith 1948, Smith, Greene and Bartner 1946, Smith and Coy 1946) have studied electrophoretically homogeneous proteins associated with immunity in the serum, colostrum and milk of the cow. Immune lactoglobulin is the predominant protein in the colostrum and also occurs to a slight extent in milk, in which it may be increased by hyperimmunization of the cow. While immune lactoglobulin and bovine serum γ globulin were quantitatively equivalent in producing anaphylaxis in the guinea pig, they differed slightly electrophoretically, in their phenyl-alanine content, and in their ultraviolet absorption spectra. The T component of bovine serum, which is also associated with immune activity, resembled lactoglobulin electrophoretically but differed slightly in its amino-acid composition. The absorbed globulin in the serum of the newborn calf possessed the mobility of colostrum globulin and not that of γ globulin (Smith and Holm 1948). As far as we are aware, no study has been made on the manner in which the concentration of antibody in the colostrum is effected. Investigations with isotopic nitrogen and known antibodies, on the lines followed by Heidelberger, Schoenheimer and their associates (1942) in the study of antibody in rabbit serum, might throw light on the manner in which the milk secreting cells may selectively concentrate and probably slightly alter the serum immune globulins, for it is hardly likely that the antibodies are formed locally by those cells.

The colostrum is of value to the young animal only if ingested soon after birth for within a few days (twenty-four hours in cattle) the alimentary mucosa becomes impermeable to its passage into the circulation. To some extent the function of colostrum may be replaced by

maternal serum given by mouth on the first day (Smith and Little 1922b). According to McDiarmid (1946) the antibody titre in the calf's serum reaches a maximum within 24 hours of colostrum ingestion and then recedes as a logarithmic curve. The period of persistence is therefore proportional to the titre of the colostrum: thus in one calf which received colostrum with an initial titre of 1:10,240 the serum gave positive results for 18 weeks, while another which received colostrum, with an initial titre of 1:640 was positive for only two weeks.

In those animals, including man and rodents, in which only a single layer of cells separates the maternal from the foetal blood, antibodies pass readily into the foetal circulation. However, it has become apparent that the situation within this group is not as simple as visualized by Mason, Dalling and Gordon (1930), for while antibodies do pass through the placenta of mice and rats the main method of transfer in these animals is via the milk (Ehrlich 1892, Berry and Slavin 1943, Kolodny 1939, Fenner 1948). Protective antibodies are present in the milk and can be absorbed by the young for about two weeks after birth. In guinea pigs and rabbits, on the other hand, mammary transmission of antibodies is of no importance. Brambell, Hemmings and Rowlands (1947) have recently shown that in rabbits maternal γ globulin and antibody pass into the yolk sac of the seven-day embryo presumably through the bilaminar omphalopleur of the yolk sac wall. Active immunization of pregnant rabbits after the fifteenth day of gestation when the bilaminar omphalopleur breaks down and disappears showed that antibodies readily entered the circulation of the embryo so that the serum antibody titres of mother and unborn young were identical. In a footnote Brambell *et al.* state that passage of the antibodies in these older foetuses is through the yolk sac splanchnopleur alone and not in detectable amount through the allantochorial placenta. These observations in conjunction with those of Kerr and Robertson (1943, 1947) with antibody to Trichomonas foetus in cattle and the results obtained by various workers on maternal antibody transfer in rats and mice,

throw serious doubt on the concept that placental permeability is inversely related to the number of layers of tissue intervening between maternal and foetal circulations in the placenta.

In humans, the colostrum and milk appear to be of negligible importance, the principal mode of transfer being transplacental; since no omphalopleur is formed in man the allantochorial placenta is the only probable route. However, all types of antibody do not pass through the placenta with equal ease. Quantitative correspondence between antibody titres in maternal and cord blood has been shown for diphtheria antitoxin (von Groer and Kassowitz 1915), tetanus antitoxin (Nattan-Larrier, Ramon and Grasset 1927), staphylococcal antitoxin (Bryce and Burnet 1932), pneumococcal antibodies (Sutliff and Finland 1932), and influenza virus antibody (Burnet and Lush 1938a). Wiener and Sonn (1946) found that "blocking" antibodies passed through the placenta readily compared with the natural or immune isoagglutinins, the former reaching the full maternal titre in the cord (foetal) blood while the latter reach a level of 1/8 to 1/16 that found in the maternal blood (Wiener and Silverman 1940). The antibodies which can be shown by the method of passive transfer to be associated with the skin-sensitive type of human allergy fail to pass the placental barrier, although protective antibodies present in the serum of treated hay fever patients were transmitted in the usual manner (Sherman, Hampton and Cooke 1940).

DIMINISHED REACTIVITY OF YOUNG ANIMALS TO TOXIC AND INFECTIOUS AGENTS AND TO ANTIGENIC STIMULI

All who have investigated the skin reactions of children to bacterial toxins have noted the absence of reaction in very young infants. This was originally assumed to be due to the presence of circulating maternal antibody, but it was shown by Okell (1932) for the Schick test, and by Cooke *et al.* (1927) for the Dick test that this failure of reaction also occurred in the infants from mothers lacking the corresponding antitoxin. Wright and Clark (1946) showed that the low reactivity of the infant's skin was not

due to insensitivity to histamine, or to inability to exhibit the typical triple response on injury. They considered that it might be due to the rich superficial vasculature of their skin, which would facilitate the escape both of injected substances and vasodilator products of injured tissues. Duran-Reynals (1942) has shown that the tissue permeability of young animals is much greater than that of mature animals. This phenomenon is not a specifically human one. Several observers have noted that young rabbits give indefinite responses to intradermal injections of staphylococcal toxin which would produce large areas of complete necrosis in older animals (Burky 1934). Infective lesions of certain types are also less obvious in young animals. The work of Pearce, Hu and Rosahn (1936) on the response to inoculation with vaccinia virus in nursing rabbits may be cited. Young tuberculous guinea pigs fail to show skin reactivity to tuberculin (Freund 1929).

It is well known to surgeons that staphylococcal osteomyelitis in infants may be associated with deceptively slight signs of local inflammation until a large region of bone is necrotic. The slight reactivity does not, of course, necessarily mean that the ultimate effect of the infection will be less in the young than in the old. It is notorious that the highest mortality from the common infectious diseases is in the first two years of life. Duran-Reynals (1940) has shown that young birds, when inoculated with the viruses of the chicken sarcomas, do not develop neoplasms, but die from a destructive non-neoplastic condition characterized by the development of "blebs" throughout the vascular system. Similar observations have been made with rabbit fibroma (Duran-Reynals 1945).

A particularly striking instance of the tolerance of immature tissues for foreign material is seen in the chick embryo on which fragments of a mammalian tumour, the Jensen rat sarcoma, have been grafted. The foreign tissue becomes vascularized from the chorioallantoic vessels and grows freely without any of the leucocytic reaction that it would provoke in an adult alien host (Murphy 1913). In some way the embryonic cells seem to be unable to

recognize and resent contact with foreign material in the way adult cells do. It is therefore not unexpected that no antibody response takes place. Grasset (1929) has shown that diphtheria toxoid injected into developing eggs confers no immunity on the chick, and that the new hatched chick also fails to be immunized.

Burnet (1941) was unable to demonstrate antibody to influenza virus in fourteen day old chicks hatched from eggs which had been inoculated on the twelfth day of incubation with living influenza virus. Here an active non-lethal infection of the embryo by an agent which is a highly active antigen in adult animals failed completely to stimulate antibody production. Jackson (unpublished) obtained similar results with bacteriophage C16 in chick embryos.

A particularly interesting example of the tolerance of foetal tissues for foreign material is Owen's (1945) demonstration of erythrocyte mosaic in calves from certain multiple births. In cows, twin young often develop a common placental circulation, and hormones interchanged between male and female twins may result in the production of "freemartins" or sterile heifers. Owen found that the common placental circulation of twin foetuses might also result in two antigenic types of red blood cell being found in both of the twins for life. One corresponds genetically to its own cells, the other to its twin's cells. The important implication of this work is that cells "foreign" to the host may be tolerated indefinitely provided they are implanted early in embryonic life.

When rabbits are to be used for the production of any type of immune serum it is the universal practice to use only adult animals, young rabbits always producing sera of lower titre. The younger the rabbit the lower the titre. According to Baumgartner (1937) precipitin produced by young rabbits, in addition to being of lower titre, shows well-marked differences from adult sera in the quantitative relations between antigen and antibody in the precipitate produced.

Wolfe and Dilks (1948) used chickens ranging in age from just hatched to twelve weeks to examine the changes

in the ability to produce precipitins with age. About half the newly hatched chicks produced no demonstrable antibody and the titres of the others were low. There was a gradual increase in the proportion of chicks responding and in the titres obtained up to the fourth week and a more rapid increase in the fifth week when the majority of the chicks inoculated gave a response of approximately adult level.

What may be a related phenomenon is the change in the character of antibodies against influenza virus A which takes place in human beings around adolescence. Children under 10 show type-specific antibodies against one or other of the human viruses, while adolescents and adults have less specific antibodies, usually acting most strongly on swine influenza virus (Burnet and Lush 1938a). It is not infrequent to find adult sera in which the only significant activity is against the swine virus.

There is abundant clinical evidence that certain human infections (e.g. typhus) have a much higher mortality in old persons than in children or young adults. This has not been related to any difference in the efficiency of antibody production in the aged, but Sabin, Ginder, Matumoto and Schlesinger (1947) found that old Japanese (more than 60 years of age) who had no neutralizing antibodies to Japanese B encephalitis before vaccination with a potent mouse-brain vaccine failed to develop either neutralizing or complement fixing antibodies, whereas children and young adults developed both these antibodies to high titres. Old individuals who had weak neutralizing antibodies (indicating past infection) but no complement fixing antibody, developed both antibodies to high titre following vaccination. The antibody-producing system of old people appeared to be effective when an anamnestic response was concerned, but failed to produce antibody when presented with a new antigen.

This brief survey of the field is greatly limited by the lack of data, but it should suggest that wide opportunities for useful research lie in this direction, and that any advances made might throw important light on the epidemiology of the infections of childhood.

CHAPTER VIII

THEORETICAL ASPECTS OF ANTIBODY PRODUCTION

Before attempting any comprehensive theoretical discussion of the nature of antibody production it seems desirable that we should give some indication of our views on the justification and utility of theoretical generalization in biological fields like the present one. In many ways immunological phenomena present the difficulties of biological generalization in a particularly characteristic form. Amongst the acceptable data of immunology are the results of chemical studies of determinant antigenic groupings such as those by Landsteiner, Goebel, Pauling and their associates. These must be discussed at the level of structural organic chemistry. But the reagents to which these determinants are bound and those with which they react are proteins in solution. The methods and concepts of organic chemistry are virtually inapplicable to proteins in solution, their scientific description is in terms of physical characteristics in solution and of the changes in such characteristics associated with changes in the composition of the solvent. One further step and we find evidence that antibody protein is derived from liquefaction of the cytoplasm of lymphocytes or plasma cells, a concept expressed in anatomical terms. The genetic aspects of immunological phenomena have become of special interest and importance from their relation to the medical problems of transfusion and their discussion introduces another category of scientific concepts. Finally, from the earliest days of medical thought the "survival value" of a capacity to become immune has been evident.

In other words, almost more than any other science immunology draws its working concepts from a whole series of categories at different levels of abstraction. Any attempt at synthesis must of necessity be an awkward makeshift but it must also be an ambitious attempt because it must

try to provide a bridge between a number of concepts developed at wholly different levels. It may even be justifiable to claim that it is precisely this multi-level character of immunology that may allow it to suggest approaches by which biological science as a whole can be wrought into a more mutually consistent pattern.

THE ESSENTIALS TO BE COVERED BY ANY THEORETICAL INTERPRETATION

If, for the present, attention is confined to antibody of classical type circulating freely in the blood, reacting *in vitro* with the corresponding antigen and giving anaphylactic type sensitization, certain conclusions can be drawn from the review of the facts of antibody production presented in preceding chapters.

(1) *Antibody is composed of globulin molecules which are produced in regions of the body where reticulo-endothelial cells, lymphocytes, plasma cells and other relatively undifferentiated mesenchymal cells are aggregated.* A first step in the production of antibody is the taking up of the antigen by cells of the reticulo-endothelial system. In some instances at least the liberation of antibody into the body fluids is a function of lymphocytes.

(2) *A second or subsequent contact with the same antigen provokes a more active production of antibody.* This is seen more clearly with toxoids than with particulate antigens, but when looked for can be observed with all types of antigens. The latent period is shortened, the antibody titre rises more rapidly and to a higher titre, and the rate of subsequent fall is slower.

(3) *Antibody in the circulation is being constantly removed at a rate which is approximately proportional to its concentration.* This is based largely on the data from passive immunity experiments with sera of the same species, but adequate reasons have been given for assuming that it holds also for actively produced antibody.

(4) *Antibody production following an antigenic stimulus rises to a peak and then diminishes, but continues at a diminishing rate often for long periods.* The classical example is the persistence of demonstrable yellow fever

antibody more than fifty years after the last contact with the virus.

(5) *Antibody production continues after the antigen responsible has been eliminated from the body.* This conclusion is still subject to controversy and is perhaps incapable of formal proof, but the weight of the evidence summarized earlier is strongly in its favour.

(6) *Antibody production is a function not only of the cells originally stimulated but of their descendants.* The cells of the reticulo-endothelial system and even more so the lymphocytes vary greatly in number in response to physiological and pathological stimuli. The average life of a macrophage is not known but for those involved in active scavenging functions it is probably not more than a few days. For lymphocytes it is usually considered to be only a matter of hours. When antibody production goes on for months or years other cells than those initially stimulated must be responsible.

(7) *The type of antibody produced varies (a) according to the species used, (b) with the age of the animal, and (c) according to the nature and frequency of the antigenic stimulus.* The change in character of the antibody following repeated reinoculation is the difference of most theoretical importance. It would indicate that an antibody-producing mechanism once established can be further modified by new antigenic contact.

Our discussion of the nature of antibody production is based essentially on these conclusions and in the first instance will be concerned only with the production of circulating antibody of classical type. It is essential that the antibodies associated with sensitization phenomena and with the phenomena of tissue transplantation should also be included, but it is preferable to consider their implications subsequently.

THE HAUROWITZ-MUDD-PAULING THEORY OF ANTIBODY PRODUCTION

In many ways the requirements for an adequate theory of antibody production and the difficulties of providing one can be most clearly seen by discussing the weaknesses

of the orthodox current theory. This is taken to be that of Pauling (1940) which is essentially a clearer statement in physico-chemical terms of the Haurowitz and Mudd hypotheses. The basis of the theory is that antibody is protein synthesized in spatial contact with the antigenically significant (determinant) atomic groupings of the antigen. In Pauling's statement antibody globulin is synthesized first by the formation of a long polypeptide chain whose constitution is common to normal globulin or any antibody globulin. This chain is then folded into a compact ovoid which represents the most stable configuration of the system, the final structure being maintained by internal hydrogen bond formation. There are many alternative methods of folding which differ little in their stability and when the folding process takes place in contact with the antigen the alternative folding which provides a complementary structure to the antigenic determinant is adopted and likewise stabilized by hydrogen bond formation. To account for the bi- or multivalence of antibody Pauling has to assume that two ends of the antibody are both synthesized against the antigen, the first end being released by thermal agitation before the other end takes up its position. It will be seen that this hypothesis premises first that the initial stage of globulin synthesis is always the formation of an uncoiled polypeptide chain, and second that the antigen is present in antibody-producing cells throughout the period over which antibody is produced.

As regards the first it seems to be a fair statement of the present position that owing to the absence of technical methods there is virtually no evidence whatever as to the nature of protein synthesis *in vivo*. Bergmann and Niemann (1937b) initiated a possible approach to the problem in their work on the breakdown and synthesis of simple peptides by various proteases. Using the action of papain on simple substances containing the —CO · NH— linkage as a model, they concluded that under physiological conditions whether synthesis or disruption of the peptide bond occurs, depends only on the nature of the groups on either side. Based in part on studies of this sort and in

part on the results of amino-acid analysis of some of the better defined proteins, egg albumin, cattle fibrin, globin and silk fibroin, Bergmann and Niemann (1937a) put forward an hypothesis of protein synthesis *in vivo* which is essentially that used by Pauling. The analytic findings for the four proteins mentioned within the limits of error of the method used indicated that there were unsuspected regularities in the number and distribution of the amino-acid residues. Their rules are that the total number of amino-acid residues is $2^n \times 3^m$, where n and m are whole numbers, and is usually equal to or a small multiple of 288 ($2^5 \times 3^2$). The number of individual amino-acid residues of each type is also $2^n \times 3^m$, where n and m may be 0, 1, 2, etc. If this is correct, in every protein each amino-acid is distributed throughout the entire peptide chain at constant intervals. To account for this regularity Bergmann and Niemann pictured a process of "spinning" a polypeptide chain by the successive addition of the appropriate amino-acids under the influence of an "organizing" proteinase.

The experimental basis of this hypothesis has been seriously questioned by Chibnall (1942) and since Bergmann's death it seems to have raised very little interest. In fact, interest in the problem of protein synthesis will probably remain dormant until some direct experimental approach is developed. In the meantime it is probably of more importance to obtain information on the structure of the simpler proteins. There appears to be increasing evidence of the existence of well defined sub-units within the conventional protein molecule and it is possible that the interesting structures now being found in some simpler natural polypeptides like gramicidin may provide the most important clues as to the nature of the secondary building stones of protein molecules.

The value of Pauling's work lies in his views on the nature of the antigen-antibody union *in vitro*. This is a subject to which physico-chemical methods can legitimately be applied and the extension of Landsteiner's pioneer work on artificial antigenic determinants has now made it possible to express in relatively precise terms what form-

erly had to be covered by the lock-key simile. For discussion of the great majority of the significant antigens used in biological work, bacteria and other micro-organisms and their components and products, it is still necessary to use such complementary pattern ideas without detailed specification since, except for one or two of the bacterial polysaccharides, the actual structure of the determinant groups is unknown.

The second basic assumption in Pauling's hypothesis, that antigen persists as a template for antibody production over the whole period during which antibody production occurs, is wholly unacceptable in the light of the evidence presented in Chapter VI. All the evidence points to the rapid destruction of foreign protein and polysaccharide within the body and the utilization or disposal of its components according to normal physiological processes. The persistence of antibody production particularly after virus infections far exceeds the time the antigen is present in the body. The only conceivable modification of the persisting template hypothesis that could be considered is that in some way the essential determinant groups are incorporated into the antibody-producing cell and kept available for template function after all the rest of the antigenic molecule has been disposed of. Apart from the rather distant resemblance to the queen bee's store of spermatozoa which, once received, lasts her a lifetime, there are no biological analogies to such a process.

There is a further serious difficulty in accepting any view of this sort. This is the rapid turnover of the cells involved in antibody production. In both physiological and pathological circumstances there is an extremely rapid turnover of lymphocytes, the average life of a cell being apparently only a few hours. In many experimental and disease conditions cells of the reticulo-endothelial system may increase enormously in number and subsequently decrease to normal. The number and relative proportions of the more or less clearly differentiated types and derivatives of primitive mesenchymal cells such as lymphoblasts and plasma cells are also highly variable. This suggests that the average life even under strictly physiological condi-

tions is short. It has already been indicated that antibody production must be a function, probably a joint function, of cells included in this general group. Where in such a rapidly changing population is the template to be kept? It cannot multiply and when a cell carrying it undergoes autolysis or phagocytosis it seems quite fantastic to believe that the template is automatically transferred to another appropriate carrier cell.

For these reasons we believe that while the picture drawn by Pauling of the physico-chemical character of the relation between the reacting groups of antigen and antibody *in vitro* is legitimate and valuable, the basic assumption that the specific configuration of each antibody molecule is produced by synthesis in physical contact with the antigen is quite untenable.

BIOLOGICAL REQUIREMENTS FOR EFFECTIVE IMMUNOLOGICAL RESPONSE

Our own approach to the problem of antibody production is predominantly biological and is based essentially on a consideration of the survival value of immunological reactivity as part of the protective mechanism against infectious disease.

It is hardly necessary to labour the fact that the capacity to acquire immunity to infectious disease has high survival value. An obvious example is to be seen in yellow fever where an infection acquired in childhood is normally mild and confers lifelong protection against any subsequent attack. A first infection in adult life is much more likely to be fatal. Quite apart from such long lasting specific immunity, there is in addition general agreement that recovery from infectious disease of any sort is to a large extent due to the activation of immunological mechanisms. In very broad terms both these desirable results are ascribed to the power of specific antibody to combine with the surface of the micro-organism concerned or with its products and so to diminish their capacity to react harmfully with the tissues and to favour their effective phagocytosis and destruction.

We may first consider the nature of the effective stimu-

lus needed to set the immunological processes in action. In nature this is the invasion of surface cells or deeper tissues of the body by micro-organisms gaining access either by their innate capacity to invade superficial cells (including those of internal surfaces) or by major or minor trauma ranging from gross wounds to mosquito bite. Antibody production results when these micro-organisms or their characteristic products pass to certain accumulations of relatively undifferentiated mesenchymal cells. These include lymph nodes and the spleen with certainty; it is also highly probable that a variety of other situations where macrophage cells are present in numbers may play a part. Examples are the omentum, the sub-epithelial tissues of the mucous membranes, the liver and the bone marrow and in some instances at least sub-acute pathological accumulations of inflammatory cells at the site of infection. The first step is almost universally regarded as the ingestion by macrophage-type cells of the antigenic particle, which may be either the living or dead micro-organism or a product of its secretion or autolysis.

Ingestion and disintegration of micro-organisms is not the sole function of reticulo-endothelial cells. Equally important is the part they play in dealing with effete or damaged body cells, the most important physiological examples of which are the taking up of red cells by the macrophages of the spleen and the phagocytosis of lymphocytic nuclei in the germinal centres of lymph nodes. It is an obvious physiological necessity and a fact fully established by experiment that the body's own cells should not provoke antibody formation. Minor exceptions to this rule concern only tissues which are "unexpendable", parts of the central nervous system and the eye. An animal's own red cells are non-antigenic. This is not due to any intrinsic absence of antigenic components; the same cells injected into a different species or even into another unrelated animal of the same species may give rise to active antibody production. The failure of antibody production against autologous cells demands the postulation of an active ability of the reticulo-endothelial cells to recognize "self" pattern from "not-self" pattern in organic

material taken into their substance. The first requirement of an adequate theory of antibody production is to account for this differentiation of function by which the natural entry of foreign micro-organisms or the artificial injection of foreign red cells provokes an immunological reaction while the physically similar autologous material is inert.

Any interpretation of antibody production along biological lines must consist essentially of a demonstration of analogies with biological phenomena in other fields. At this stage it is worth considering what these other fields are. Antibody production represents a *change* in a physiological response induced by a chemical stimulus. We have given cogent evidence that the changed capacity to respond is transmitted to cells descended from those in which it was induced. There is not the slightest evidence of chromosomal inheritance of acquired immunity. The fields in biology then, in which useful analogy may be sought, are those similarly concerned with circumstances in which a chemical stimulus can permanently or semi-permanently modify the reactivity of a cell and its descendants. The formation of adaptive enzymes by micro-organisms and the processes of embryonic differentiation as controlled by organizers immediately come to mind. The induction of mutation by mustard gas, of chromosome duplication by colchicine and of malignancy by carcinogenic hydrocarbons appears to be more remote. The development of drug-resistance by micro-organisms is generally regarded as a process of selection amongst spontaneous mutations but Hinshelwood (1946) has brought evidence that in addition processes akin to the appearance of adaptive enzymes may be concerned. Finally there is the development in mammals of tolerance to increasing doses of non-antigenic poisons such as arsenic or morphine to be considered.

ADAPTIVE ENZYMES

Before developing the thesis that the most fertile analogy is with adaptive enzyme formation, it will be advisable first to outline the general character of adaptive enzyme development in bacteria and yeasts.

It is often found that if a bacterium or yeast is transferred to a medium containing a sugar which it does not normally ferment it will, after a variable period of time, develop the capacity to ferment the new sugar. This change is usually not seen unless the population of micro-organisms is able to multiply but in a number of well established instances washed resting bacteria or yeasts can after a few hours develop such a capacity. This appearance of new fermenting capacity is ascribed to the formation of an adaptive enzyme. It has often been pointed out, however, that there is no sharp line to be drawn between the two types; Spiegelman (1945), for instance, points out that *E. coli* in the absence of sucrose may show an invertase activity of 12-40 units, but in the presence of the substrate reach a level of 450 units.

Monod (1947) in a recent review summarized the conditions in micro-organisms as follows—

(1) The formation of most enzymes attacking exogenous substrates is specifically increased in the presence of the specific substrate.

(2) In many cases no appreciable enzyme formation occurs in the absence of the specific substrate.

(3) Enzymic adaptation is as highly specific as enzymic specificity.

(4) Enzymic adaptation occurs only in growing cells or at least does not occur under conditions preventing protein synthesis.

In yeasts, the capacity to develop a given type of adaptive enzyme can be shown to be a genetic character, determined by a single gene. The appearance and quantitative development of the adaptive enzyme, however, depends on the presence of the substrate and once produced the enzyme appears to be free of direct genic control. Spiegelman, Lindegren and Lindegren (1945), showed that when two yeasts, one capable of producing an adaptive enzyme acting on melibiose, the other lacking this capacity, were hybridized, the hybrids were active in this respect. When haplophase cultures derived from asci produced by the hybrid were tested the results differed according to whether or not all the cultural manipulations

had been made in the presence of melibiose. In its absence equal numbers of active (i.e. capable of developing melibiase) and inactive cultures were obtained according to the normal genetic rules. In the presence of melibiose a marked excess of active cultures was obtained. The implication was that an adaptive enzyme once established in the cytoplasm could persist there, despite nuclear segregations which would forbid its primary appearance. To persist in these circumstances demands that the adaptive enzyme must in some sense be a self-reproducing unit. These experiments have not been fully confirmed and Spiegelman (1948) has recently discussed possible reasons for the non-producibility of the phenomenon. Undoubtedly more than the simple continuing presence of the substrate is necessary but the additional factors have not been defined. Even in the early experiments some deviations from the normal Mendelian inheritance were observed in the absence of substrate and Spiegelman discusses the possibility of transference of "plasmagenes" from adapted cells to genetically negative cells, so converting them to the phenotypically positive character. Monod (1947) insists that failure to confirm these observations removes the only evidence so far provided that any enzyme constitutive or adaptive is not under direct genic control. In our opinion, however, there is sufficient evidence for the existence of cytoplasmic self-replicating entities, e.g. the kappa component in "killer" strains of Paramoecium (Sonneborn 1946, 1947), the melanin units of the chromatophores in guinea pig skin (Billingham and Medawar 1948) and probably the Rous sarcoma "virus", to feel that it may be possible to define the conditions under which the Lindegren experiment will be regularly reproducible. Quite apart from these considerations there is much indirect evidence to suggest that a self-replication process is concerned in the increasing fermentative activity shown in phenomena of this general type. This point of view is whole-heartedly adopted by Hinshelwood (1946) in his discussion of the problems of bacterial growth. A comment by Delbrück (1945) in a discussion of Lindegren and Spiegelman's work indicates clearly the general type

of modern speculation on these problems. He considers a gene G is stable so long as it remains in the nucleus: it produces there an enzyme E similar to itself. When this enzyme passes to the cytoplasm it becomes unstable but can be stabilized (and perhaps modified) by contact with appropriate substrate. When so stabilized it becomes a self-replicating unit which in the cytoplasm represents and replaces the gene.

Hinshelwood's (1946) discussion of bacterial adaptation is concerned almost wholly with the behaviour of large bacterial populations and tends to minimize the importance of mutation and selective survival. Within these limitations, however, his results show very interesting analogies to some aspects of antibody production. His statement of the principles of bacterial adaptation to drugs, e.g. sulphonamides, or to new sources of carbon may be quoted:

(1) Adaptation may be complete without being stable against reversion.
(2) The longer the training process the greater the stability.
(3) Reversion may be partial in the sense that the reverted cells retain some of the acquired property.
(4) Stability of the most thoroughly adapted cells is not absolute. (One might comment that this could probably be said with equal truth about any such quality of "normal" cells.)
(5) Training usually imparts the new character without impairing any of the old.

A further observation from Hinshelwood which may suggest a partial interpretation of the production of antibody to very "poor" antigens by the use of adjuvants may be added. Sometimes a bacterium may fail completely to utilize a substance B as an alternative for A when the change is made abruptly, but will become adapted by continuous growth in mixtures of A and B in which the proportion of A is gradually reduced.

For obvious reasons the study of adaptive enzymes has been confined to unicellular organisms. It is not practical to subject the cells of an intact animal to an abnormal concentration of some substance that might be expected

to provoke the appearance of an adaptive enzyme. However, there is an indirect approach that strongly suggests that phenomena analogous to adaptive enzyme formation do occur in animal cells. This is simply the differentiation which takes place during embryonic development. From the fertilized ovum in which there are certainly no high concentrations of specialized enzymes there develop cells such as those of the different glandular organs with high content of enzymes or proenzymes.

The modern development of biochemical genetics, particularly under the stimulus of Beadle and his colleagues, is tending increasingly to equate gene action with enzyme action. Very numerous examples are now available in organisms ranging from Neurospora to man in which a single gene mutation or loss is associated with failure of a single defined enzyme activity in all cells of the phenotype. The several types of human abnormality associated with failure to metabolize tyrosine normally (albinism, alcaptonuria and the type of mental defect associated with absence of phenylpyruvic oxidase) provide the best known examples. A much more extensive material is available in the Neurospora mutants of Beadle's classic work.

As a working hypothesis to cover (in the first instance) biochemical mutations of this type, Beadle (1945) has suggested that the relation of enzyme to gene is one of direct descent. The gene replicates at each cell division to produce a like gene but an additional process is postulated by which the gene produces a replica or partial replica which passes into the cytoplasm. This partial replica undergoes further replication to an extent determined by the type of differentiation undergone by the cell in question to produce the required concentration of enzyme molecules. It seems more than likely that a process closely related to, if not identical with, adaptive enzyme formation in bacteria must play a part in the progressive shaping and specialization of intracellular enzymic functions as differentiation proceeds.

PROTEIN SYNTHESIS IN VIVO

Since all known enzymes are proteins this view of the

relation between genes and enzymes immediately raises the question of the mode of protein synthesis. Bergmann and Niemann (1937a) explained the apparent regularities in the structure of the polypeptide chain of the proteins which they studied, as resulting from the functioning of an "organizer" in some way analogous to the organizers concerned with the early differentiation of the amphibian embryo. They considered that the organizers would be found amongst the intracellular proteinases (papainases) which alone of known enzymes have some of the required capacities. They can break down proteins in part at least to amino-acids and they are also capable of synthesizing peptide bonds under the same environmental conditions of temperature and pH as are optimal for their proteolytic activity.

The intracellular enzyme was pictured as having at its disposal a number of protein fragments of different size and structure; it subjects these fragments to a series of transformations by synthesis, hydrolysis and replacement until there is produced a protein pattern which is stable in the presence of enzyme. This seems to demand an extreme specificity of enzyme function when one considers the enormous number of proteins in existence. The intracellular proteinases which are capable of constructing a complex functioning protein must themselves be at least as complex, and there is no avoiding the suggestion that perhaps the proteinases are merely the living protein itself. Bergmann and Niemann do not consider this possibility in regard to normal protein synthesis, but state: "If the proteinases are themselves proteins and at the same time have the ability to synthesize other individual proteins, then there must exist proteinases which have the ability to synthesize replicas of their own structural pattern and therefore are able to 'multiply' in suitable surroundings . . . it would appear desirable to investigate (tobacco mosaic virus protein) and other viruses for possible proteinase activity." Our earlier comment (1941) on this was that it might be incorrect to assume that self-synthesizing proteinase should necessarily have a virus type of activity —another equally legitimate view would be to regard the

great majority of the living protein structures as themselves self-synthesizing proteinases. This concept was then used as a basis for the development of a general theory of antibody production.

In the period since 1940 interest in the problem of intracellular protein synthesis has developed in a rather unbalanced fashion. The chief experimental development has been concerned with the use of histo-chemical methods for the detection and rough measurement of localized concentrations in the cell of ribo- and desoxyribonucleic acids, and of alkaline phosphatase. The concept is growing that high concentrations of ribonucleic acid and of alkaline phosphatase in the cytoplasm of a cell is indicative of active protein synthesis in the cell. It is also becoming evident that granular intracellular structures including the mitochondria represent highly concentrated accumulations of enzymically active material including nucleoprotein (ribonucleic acid type), alkaline phosphatase, cytochrome oxidase, succinic dehydrogenase and adenylpyrophosphatase. It seems likely that these granules are centres responsible for providing the energy and material for protein synthesis including necessarily antibody globulin synthesis.

Studies with radioactive tracer elements have established the intensely dynamic character of intracellular chemical structure including proteins, but have not yet provided an approach to their mode of synthesis. Methods for the polymerization of amino-acids into protein-like complexes *in vitro* are being developed and it may be that this approach will lead to ideas that can be applied to the natural intracellular synthesis. It is probably fair to state the present position as: (a) From the chemical point of view virtually nothing is known of the details of protein synthesis *in vivo*; (b) there is an increasing tendency for biologists to use explicitly or implicitly the conception that significant intracellular proteins, particularly enzymes, are synthesized by a process of replication (an essentially biological concept not yet expressible in chemical terms).

Monod (1947), for instance, states that "the formation of these highly complex and highly specific molecules

(enzymes) must involve a sort of prototype mechanism where the configuration being formed is determined and defined by a pre-existing 'master pattern' ". He considers it likely, however, that only a small element of the specific molecule need be represented in the master pattern, whether this is in the gene or in the cytoplasm.

Weiss (1947) has even gone so far as to speak of molecular ecology by which the cell is viewed as a complex population of molecules and molecular groups. The cellular organization then becomes the resultant of interaction, competition, selective increase (proliferation) and regrouping of the units.

An extension of this general point of view is to regard those proteins which are not functioning components of living cells as being "partial" replicas of an appropriate "master pattern" molecule. These differ from the prototype in lacking the capacity for further replication either intrinsically or because they are not appropriately related to other cellular components. In this group would be found structural proteins like collagen, reserve food stores like egg albumin and the yolk proteins, glandular secretions like mucin and the digestive enzymes and the proteins of blood plasma. We may summarize our interpretation of the processes of protein synthesis and cellular differentiation in relation to the problem of antibody production in some such schema as that in Figure 11.

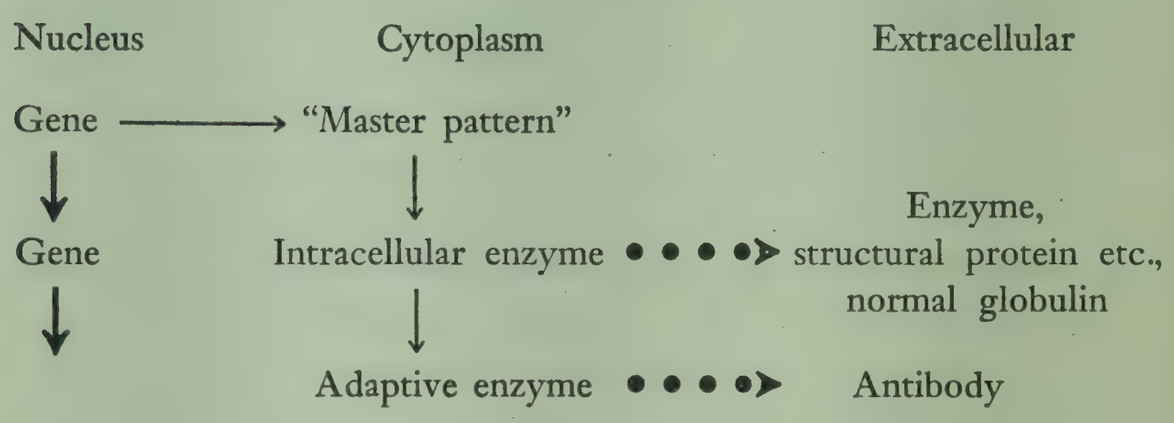
On this view there is a progressive series of changes in gene products, each being related "genetically" to the preceding one, but each being modified by its new internal environment in a direction appropriate to the functional activity of the cell involved.

There appear to us to be only insignificant differences between the schema outlined above and Northrop's recent discussion of the nature of protein synthesis (Northrop, Kunitz and Herriot 1948). The primary gene derivative for which we have used Monod's term "master pattern" corresponds to Northrop's proteinogen except perhaps in that we are inclined to think that there must be a proteinogen corresponding to each relevant gene instead of the small number postulated by Northrop. As indicated

beneath Figure 11, all the processes which are hypothecated will require the provision of the necessary energy and "bausteine" which, as Northrop points out, need not necessarily be amino-acids.

FIGURE 11

Schematic Representation of Protein Synthesis and Antibody Production



Heavy arrow: replication of pattern.
Light arrow: partial replication of pattern.
Dotted arrow: liberation of protein unit bearing specific pattern but lacking any further capacity for replication.
All processes will necessarily be dependent on intracellular mechanisms for supply of energy and "bausteine".

THE NATURE OF ANTIBODY PRODUCTION

On the basis of this preliminary discussion it is possible to state explicitly the interpretation of the nature of antibody production which we at present favour. There is a physiological mechanism mediated by cells of the reticulo-endothelial system, plus in all probability a variety of other relatively undifferentiated mesenchymal cells including lymphocytes and plasma cells, concerned with the breaking down and disposal both of effete or damaged body cells and of foreign predominantly micro-organismal material. Autologous material is dealt with by appropriate enzymic systems without involving change from the existent status. Foreign organic material is dealt with by the same cells and processes but at some point in the mechanism a modification is necessary to allow what, for example, was effect-

ive in dealing with blood group polysaccharide, to deal with a bacterial polysaccharide. An appropriate adaptive enzyme is produced which has the necessary configuration for effective adsorption to the unfamiliar substrate. It is immaterial from the point of view of the theory which particular histological cell type is responsible for the enzyme action involved, or at what stage in the breakdown of the substrate the specific adsorptive relationship is necessary.

It is important not to press the analogy with known adaptive enzymes too closely. Those which have been investigated are concerned with the destruction of simple molecules and are strictly limited in the number of new substrates to which they can be adapted. If according to current usage one confines the term enzyme to the agent responsible for some chemically defined action on a particular aspect of the substrate molecule, it is inappropriate to refer to the postulated changes initiating antibody production as the formation of an adaptive enzyme. But it is the closest analogy we can provide. It may well prove more correct to suggest that what we are considering is a reorganization within the cell of a rather complex mechanism which functions as a preliminary to a series of enzymic actions on the antigen substrate. The unit concerned may for instance have to effect some form of fixation of the antigenic molecule or particle before disintegration by intracellular enzymes occurs. This would allow for the possibility that in some instances substances incapable of being broken down into metabolizable components might be antigenic. As indicated in Chapter VI there is evidence that some of the specific pneumococcal polysaccharides may fall into this category. The essence of our hypothesis is that, irrespective of whether it is legitimate or not to call it an adaptive enzyme, a new self-replicating system is now present in the cell which can be caused to multiply by the appropriate stimulus. From the nature of the phenomenon of the accelerated secondary response to an antigen it seems highly probable that the function of first antigenic contact is to produce the adaptive modification while subsequent contacts stimulate its replica-

tion and the eventual liberation of partial replicas into the blood stream. The hypothesis also demands that when cells carrying the adaptive enzyme multiply the descendant cells will also carry the new character.

Rather direct support for this view is provided by the experiments of Dougherty, Chase and White (1945) who found that the cells of a transmissible lymphosarcoma of mice could produce antibody. If cells from an immunized animal were transferred to another with the development of the tumour, antibody was liberated into the circulation and the same result followed transplant to a third generation. This result was confirmed by Price in Northrop's laboratory (Northrop, Kunitz and Herriot 1948).

Circulating antibody of classical type, i.e., antibody responsible for *in vitro* aggregation reactions with appropriate antigenic molecules or particles, is regarded as a partial replica of the intracellular enzymic system in the sense of carrying the specifically modified adsorptive pattern of the enzyme but lacking both enzymic activity as such and also the capacity to replicate itself. In these respects it is entirely analogous to the egg albumin secreted by the avian oviduct, or any other secretion of extracellular protein.

In the course of work in an entirely different field we have encountered an analogous phenomenon which may make the concept clearer. Influenza viruses in their action on red cells make use of two dissociable mechanisms. There is first a relatively specific adsorption to the cell surface, the cell component concerned being a muco-polysaccharide. For viruses like mumps and Newcastle disease minor enzymic damage to the polysaccharide renders adsorption impossible. For some other viruses of the group much more extensive alteration of the polysaccharide receptor is necessary before adsorption fails to occur. The second step is an enzymic modification of the receptor to which the virus is adsorbed; this proceeds only so far as will render the receptor incapable of adsorbing the virus. As soon as this happens the virus is released and enzymic action automatically ceases. Something very nearly equivalent to the partial replica (antibody) postulated above

can be obtained by simply heating the virus to an appropriate temperature. Such heated virus is adsorbed to red cells according to the same rules as unheated virus but it has completely lost any enzymic action on the receptor.

Incidentally it may be pointed out that this enzymic action of influenza virus is one of the few examples available in which a relatively specific adsorption is required before enzymic action occurs. It seems highly probable that many other enzymic activities have a similar quality but technical methods for demonstrating it are not available. The influenza virus example thus seems to provide some support by analogy for the intracellular process postulated as the basis of antibody production.

CELLULAR ASPECTS OF THE IMMUNOLOGICAL RESPONSE

The evidence in regard to the respective parts played by macrophage, lymphocyte and plasma cell in antibody production has been discussed in Chapter VI. It is necessary here to consider how this work can be correlated with the general hypothesis of antibody production that has been expounded.

Since the evidence in regard to antibody production in lymph nodes is more precise than for any of the other sites, discussion will be centred on this aspect. We would regard the lymph node as a repository of highly reactive primitive mesenchymal (reticulum) cells which under appropriate stimulation can proliferate and take on one or more of several functions. The function adopted will determine the histological appearance of the cells involved. There is a subtle but probably real difference between this statement and the more orthodox one that proliferation of reticulum cells gives rise to genetically determined cell types each with specific functions.

The macrophages are responsible for taking up the antigenic molecules or particles and in them the initial stages of antibody production take place. The plasma cell is a cell in which rapid production of antibody (or possibly in some circumstances, non-specific or pathological) globulin is taking place.

As Thorell (1947 a and b) points out, the characteristic

staining reaction of the plasma cell cytoplasm is due to a high content of ribonucleotides and this is regularly associated with active protein synthesis. Irrespective of whether they are descendants of macrophages which have taken up antigen or of reticulum cells to which an antibody-producing stimulus has been transferred, the plasma cells are such in virtue of their active cytoplasmic synthesis of protein. We consider that all the evidence is consistent with the view that the *rapid early production of antibody following especially a secondary stimulus* is a function of those cells which in stained smears and sections appear as plasma cells or as forms intermediate between the primitive reticulum cell and the typical plasma cell.

There is valid experimental evidence to show that some antibodies at least are liberated into the circulation by the partial disintegration on cytoplasmic liquefaction of the cells (plasma cells and lymphocytes) produced in and found in the lymph proceeding from the lymph nodes draining the area in which the antigen was deposited. Interpretation of this finding in terms of our general hypothesis might follow either one of two lines.

(1) The antigen is taken up by the reticulo-endothelial cells (macrophages) and the self-replicating immunologically adapted enzyme which for brevity may be called the primary unit is established in these cells. Plasma cells may, as Fagraeus believes, be produced by multiplication of the macrophages or they may receive primary units from them by a mechanism analogous to that described by Billingham and Medawar (1947, 1948) for melanin production in the chromatophores of parti-coloured guinea pig skin. Conversion of macrophages to lymphocytes seems very unlikely but the same two possibilities would have to be considered for them. On the second supposition, transfer of the primary unit from one cell to another, the plasma cells and lymphocytes would serve as "nurse" cells in which the primary units find appropriate conditions for the production of partial replicas.

(2) The reticulo-endothelial cells may be responsible only for a preliminary disintegration of the antigenic particle, the actual antigenic pattern being then transferred

to lymphocytes or plasma cells in which the primary unit is formed and all subsequent processes occur. The latter is the conception favoured by American workers. Our own preference is strongly toward one or other form of the first alternative mainly because of the necessity of having prolonged conservation of the primary units.

Taking a clue from White and Dougherty's work that dissolution of lymphocytes under hormonal action results in an anamnestic antibody response, we feel that the lymphocyte's part may be to maintain the slow liberation of antibody that goes on for years after the antigenic stimulus has ceased. If we take a lymph node subjected to an intense antigenic stimulus so that virtually all macrophages lining the sinuses take up the antigenic particles, our interpretation would picture the transfer of a potential antibody-producing stimulus from such macrophages to most or all of the stem cells in the node. Many of these would proliferate and develop toward plasma cells from which most of the acutely produced antibody would derive. Others would give rise to lymphocytes in which cytoplasmic activity was much less marked. Still others would remain quiescent or give rise to cells like themselves. We can conceive no way in which antibody-producing power can persist far beyond the individual life of the "end-cells" which produce it unless the capacity is in some way implanted in the mother cell type from which the expendable cells are descended.

This interpretation must be purely a tentative one. As for any alternative, the evidence for it is almost wholly indirect. It is hard to see how the application of currently available techniques could be used to establish directly which cells are responsible for antibody production. Probably the answer will come from the development of finer biochemical techniques for the study of intracellular physiology. We feel that a clarification of the function of the lymphocyte would probably throw a great deal of light on the general problem of immunity.

Our discussion of the cellular aspects of the theory has been concerned essentially with the response to particulate antigens reaching the lymph node from a peripheral site of infection or inoculation. It would be equally ap-

plicable to conditions in the spleen when the antigen circulates in the blood. Practically nothing is known, however, about the site of antitoxin formation and even less about the cells concerned in the process. We have described in Chapter VI experiments showing the absence of antitoxin production in the lymph node after a secondary inoculation of staphylococcal toxoid. It may be, as Buttle's (1934) experiments suggest, that antitoxin production is a function of cells widely distributed throughout the body rather than of the specialized accumulations of cells in spleen and lymph nodes. It would be equally unwise to assume that such agents as the natural isoagglutinins, the Wassermann antibody and the circulating reagin of the allergic subject are produced in the same fashion as bacterial agglutinins. The facts for each type of antibody will have to be determined and until this is done further speculation is probably useless.

THE "SELF-MARKER" CONCEPT

In the first section of this discussion the importance of a means by which the same functional system of cells could react in two sharply different ways toward effete and damaged body constituents on the one hand and foreign organic matter on the other was stressed. In a recent review of the relations between immunology and genetics (Burnet and Fenner 1948) the implications of this necessity were analysed and the hypothesis put forward that differentiation is based on the existence of a small number of marker components in the expendable body cells. It seemed to us quite inconceivable that every complex molecule in a red cell or a lymphocyte should be so labelled that it can be recognized as autologous by every macrophage of the body and therefore provoke no antibody response. The alternative which was suggested during an attempt to analyse the significance, genetic and otherwise, of the serological differences between red cells of related races or species, is that only a limited number of recognizable components need be postulated. There may be 5 or 10 marker components in a red cell but probably not many more. In a human red cell one of them at least

is of polysaccharide character (the ABO blood group substance). It is our contention that the primary units (adaptive enzymes) on which antibody production is based are modifications of enzyme systems primarily adapted to specific adsorption to one or other of the self-marker components of the body cells. When, and only when, specific adsorption occurs the marker is enzymically destroyed without disturbance of intracellular equilibrium. Keeping the blood group polysaccharide as our typical marker we can consider what happens when a chemically related but structurally dissimilar polysaccharide, e.g. from a foreign red cell or an invading pneumococcus, enters the cell. The same general type of enzymic action is called for but the new substrate does not quite fit the specific adsorptive pattern of the enzyme. If the pattern of the enzyme can be adapted to fit the substrate configuration then the primary unit of our hypothesis has been established and the process leading to antibody production set in train.

On this view there are as many potential types of antibody as there are enzyme units tuned to particular marker components in those cells capable of initiating the production of antibodies. It may be found that such units are differentially distributed in different cell systems of the body rather than, as we are tacitly assuming, that each is present in all cells of macrophage type. Almost certainly there is a significant difference of this sort between cells within the C.N.S. and those elsewhere in the body and the concept must be left flexible enough to accommodate the results of future studies on pathological conditions which may be due to "auto-antibody" production foreshadowed by those of Kabat, Wolf and Bezer (1947) and Morgan (1947b).

On general biological grounds one would presume that the antigen most effective in antibody production would have a determinant pattern distinctively, but not too distantly, removed from the marker pattern. A very minor difference might not act as a stimulus to adaptation and too remote a resemblance might make adaptation impossible. Where adaptation is difficult this probably means that only in a very small proportion of contacts made under

the most effective conditions of orientation, etc., can complete or partial modification be induced in the enzymic unit. In Chapter IV the evidence has been provided that progressive changes in antibody can be produced on continued immunization with the same antigen. This makes it possible to conceive that a substance which, owing to its great disparity from the nearest self-marker pattern, is a very poor antigen, may, if it is continually presented to the same cells over a long period, modify the appropriate enzymic unit by stages until an effective antibody is eventually produced. The function of the "water-oil emulsion" adjuvants is most likely to allow such a prolonged delivery of antigen from the local depot to the antibody-producing cells.

DEVELOPMENT OF "MARKER" RECOGNITION IN EMBRYONIC LIFE

It is of considerable interest that foetal mammals and chick embryos are incapable of producing antibody (Grasset 1929, Burnet 1941) and the full capacity to do so develops only slowly in the young free-living animal (Wolfe and Dilks 1948). This raises the suggestion that the process by which self-pattern becomes recognizable takes place during the embryonic or immediately post-embryonic stages. The hypothesis of antibody production that we have developed can readily be adapted to such a possibility. Taking the red cell as the typical expendable body cell we may assume that in embryonic life phagocytosis and disintegration of worn out cells is actively taking place. During this phase appropriate intracellular enzyme systems are being adapted to deal effectively with those components which need to be broken down for reintegration into the metabolic activities of the body. One of the components so dealt with is doubtless the polysaccharide responsible for "blood group specificity" in some and perhaps most species. Some phase of the enzymic process concerned is in this period progressively adapted to deal with polysaccharide of this particular pattern. An adaptive enzyme specifically uniting with and initiating the destruction of the component is brought into being

in the cells that destroy erythrocytes. This newly patterned enzyme becomes stabilized as part of the inheritable structure of these cells and is transmitted indefinitely to their descendants.

With the development of the free-living state this lability of intracellular enzymes is lost, the patterns engraved during embryonic life harden as it were and become permanent possessions. In such a process we have envisaged something essentially similar to the process of antibody production except that the subsequent steps leading to the appearance of free antibody do not occur.

This conception may be of crucial importance for the acceptance or not of the present theory of antibody production since it has important implications which are susceptible to direct experimental or observational proof.

(1) If in embryonic life expendable cells from a genetically distinct race are implanted and established, no antibody response should develop against the foreign cell antigen when the animal takes on independent existence. What is at present a unique natural example of such a circumstance has been described. In the course of detailed studies of blood groups in cattle and their inheritance, Owen and his colleagues (Owen 1945, 1946, Owen, Davis and Morgan 1946) studied examples of multiple births in which there had been a common placental circulation although the embryos were of multiovular origin. In these instances the normal segregation of blood group character did not occur. Two calves from the same birth would each show two coexisting serological types of cell when by ordinary genetic rules each should have one only. Under normal circumstances the blood cells of one calf should have been antigenic if injected into the circulation of the other; when the cross transfusion takes place in embryonic life no immunological response follows either then or subsequently. A very interesting field for direct experimentation is opened up by this finding, particularly if the same type of phenomenon can be induced by intravenous inoculation of foreign embryonic blood cells in chick embryos.

(2) It would also be expected that following a

generalized non-fatal infection by a pathogenic micro-organism of the embryo *in utero*, the animal after birth would be incapable of responding with antibody production to injection or infection with the same micro-organism. In nature such occasions are naturally rare or at least unrecognized, but the condition is known to occur in certain strains of mice infected with lymphocytic choriomeningitis virus. In work extending over several years, Traub (1936, 1938 and 1939) carried out a comprehensive series of studies of the natural history of this disease in an infected stock. At the close of his studies the infected community of mice appeared to have reached a virtual state of symbiosis with the virus. All mice were carriers for the greater part of their lives and all young mice were infected *in utero*. No clinical evidence of illness was apparent and the mice were quite resistant to intracerebral challenge with virus. No neutralizing antibody could be detected in the blood nor any complement fixing antibody.

Adult mice from healthy uninfected stock could be infected by various routes and after recovery remained carriers for a variable period. Subcutaneously infected mice in general had only a very brief carrier period and developed partial immunity which became complete once an intracerebral challenge dose of virus had been withstood. The partial immunity was manifested by an accelerated appearance of symptoms on intracerebral test inoculation, suggesting a sensitization reaction. Both the partial and the subsequent complete immunity were unassociated with continuing infection. Little or no virus neutralizing antibody was present in the serum of such firmly immune animals but, unlike those who remained carriers, complement fixing antibody was regularly detectable.

These phenomena are obviously complex but there is the development of a tolerance to the foreign micro-organism during embryonic life which is in line with the present hypothesis.

Non-lethal infection of chick embryos with viruses does not result in any inhibition of the capacity of the chick

to produce the corresponding antibody after hatching. Fox and Laemmert (1947) found that chick embryos infected with yellow fever virus might hatch and subsequently show circulating antibody. In all probability all the embryos they used were infected. Those which had virus circulating at the time of hatching developed antibody, those with no virus demonstrable did not. Recent experiments (Burnet and Stone, unpublished) with influenza viruses are comparable to those of Fox and Laemmert in that a small proportion of chicks infected *in ovo* showed low titre antibody when bled at 4-6 weeks after hatching. Those showing no antibody responded normally to an intravenous injection of virus. Similar absence of effect or the subsequent immune response was shown when non-infectious antigens, bacteriophage and mammalian red cells were inoculated into the embryo. It is clear that something more than mere casual presence of the antigen in the embryo is necessary if subsequent immunological reactivity is to be modified in the way we have suggested.

SUMMARY

This concept of the nature of antibody production is a logical extension of the concepts now being developed in various fields of the nature of the processes of cellular differentiation. The nuclear complement of genes is constant for every somatic cell of the body, the cytoplasmic components, derived in the last analysis from genic pattern, change qualitatively and quantitatively in the course of embryonic development according to their temporal and spatial distribution. The functional cytoplasmic entities (which may be termed plasmagenes, enzymes or living protein) are self-replicating but subject to modification by the circumstances of the changing intracellular environment and particularly according to the concentration and qualitative character of their appropriate substrates. In warm blooded vertebrates at least, and probably to some extent throughout the Metazoa, there are functional units concerned with the breakdown of complex molecules which form part of the expendable

cells of the body. In embryonic life these units become specifically adapted to the characteristic patterns of a number of the molecules concerned. This can be expressed as the implantation in the "scavenger" cells of the body of a means of recognizing "self-marker" components of expendable body cells. With birth or hatching the body is exposed to the entry of foreign organic material; this is dealt with by essentially the same processes as are applied to expendable body cells. The intracellular enzymes concerned in dealing with self-marker components are labile enough to take on a new adaptive pattern fitting them to deal with foreign substances of the same general character but of different molecular pattern. These secondarily modified units have the normal capacity to replicate, accentuated and modified by the evolutionary development of their function as protectors against disease. According to circumstances they may be transferred to other cells or give rise to partial replicas carrying the specific complementary pattern but not the enzymic activity. These partial replicas are the globulin molecules of circulating antibody.

In our discussion no attempt has been made to indicate how the antibody pattern complementary to that of the antigen is impressed upon the intracellular unit. Pauling's suggestion that many different types of folding of a polypeptide chain are possible without significant differences in free energy may point to the type of alteration induced. But it is necessary to remember that the major requirement of the modified pattern is that it should be capable of replication as such. No chemist has so far gone further than to recognize the difficulty of any approach at the chemical level to the basic biological phenomenon of the replication of organic pattern.

CHAPTER IX

ANTIBODIES INVOLVED IN SENSITIZATION PHENOMENA

In the previous chapters we have been concerned with the processes by which classical circulating antibodies are produced. In suitable experimental animals it can be shown that the development of some hypersensitive tissue reactions to an antigen is directly correlated with the appearance of circulating antibody and there is fairly general agreement that all or most types of acquired hypersensitivity are due to the localization of antibody in the reactive tissues. In this chapter we are concerned, first, with the evidence for the existence of antibodies responsible for tissue sensitization and, second, to discuss the bearing of sensitization phenomena on the general theoretical approach to antibody production outlined in the preceding chapter.

There are four recognizable clinical types of sensitization reaction which may be labelled the anaphylactic, hay fever, contact dermatitis and tuberculin types of sensitization. The divisions are not clear cut and there is much to be said for Chase's (1948) suggestion that only two major divisions should be recognized.

His first group is characterized by a rapid inflammatory response appearing within a few minutes after the injection of antigen ("immediate-type" response). Another feature of this type of reactivity is the presence of circulating antibody which can be used to transfer sensitivity to a normal animal of the same or a related species. This group includes typical anaphylactic sensitization, including Arthus type reactions in the rabbit and the various types of human sensitization giving the "triple response" to intracutaneous injection of antigen.

The second group ("delayed-type" response) shows a reaction reaching its maximum 24 to 48 hours after the injection of antigen. It includes contact dermatitis or

eczematous sensitization and various reactions of the tuberculin type. No circulating antibody can be demonstrated but under suitable conditions the reactivity can be passively transferred by exudate cells of the sensitized animal.

Group 1. Anaphylactic sensitization is taken to include serum sickness in man as well as the classical Theobald Smith and Arthus phenomena in guinea pig and rabbit respectively. In these conditions the sensitizing antibody appears to be identical with precipitin and is of the classical type giving aggregation reactions with the antigen *in vitro* and in human being passing freely through the placenta to the foetus.

Hay fever type sensitization differs mainly in the character of the associated circulating antibody which can be demonstrated by the Prausnitz-Kustner reaction. Compared with the anaphylactic antibody this is much more thermolabile, produces no aggregation reaction *in vitro*, is much more readily fixed in the tissues and fails to pass the placenta.

Group 2. Eczematous sensitization (contact dermatitis) is characteristically produced by treatment or natural exposure of the skin to simple chemical compounds, such as dinitrochlorobenzene or picryl chloride, which combine readily with protein. This type of sensitization is not passively transferable by serum but in the experimentally sensitized guinea pig there is frequently present a serum antibody which by the Prausnitz-Kustner procedure will transfer a sensitivity of the "immediate" type to a normal guinea pig. Chase (1948) considers that two different sensitivities arise in parallel, the relative preponderance of "immediate" or "delayed" type sensitization depending principally on the chemical character of the effective substance but probably also in part on the route by which sensitization is induced. Eczematous sensitization is normally only produced by treatment of the skin and can only be demonstrated by application of the sensitizing agent to the skin. Raffel and Forney (1948), however, have recently shown that typical and high grade eczematous sensitization can be produced in guinea pigs by

intraperitoneal injection of picryl chloride plus purified wax from tubercle bacilli. They suggest the possibility that the lipids as well as the proteins of the skin may play a part in rendering the sensitizing agent immunologically active.

Tuberculin type sensitivity has many points in common and in view of Raffel and Forney's work the chief distinction, that sensitization can be produced by a tuberculous lesion anywhere in the body, loses most of its importance. Most other types of micro-organism which produce chronic or sub-acute lesions can give rise to a similar type of sensitization, usually of lesser intensity than the tuberculin reactivity.

Owing to the technical difficulties in studying antibodies which either fail to appear in demonstrable quantities in the blood or when they are present are not measurable by *in vitro* techniques, there is much less experimental evidence available in regard to the mode and site of origin of the antibodies concerned. Some would even raise doubts as to whether any agent that can legitimately be called an antibody is involved in the process of sensitization of the "delayed" type. A further difficulty arises in regard to most of the examples which have been extensively studied in that the antigens concerned, pollen grains or micro-organisms, are highly complex living cells containing a variety of potential antigens. It may be very difficult to establish how many and which components are concerned in the particular type of immunological reaction being studied. It will be convenient to take the available evidence in regard to each of the main clinical types of sensitization. The anaphylactic type requires no separate discussion as far as production of the antibody is concerned.

Almost the only relevant evidence in regard to the hay fever type of sensitization is concerned with the fact that parenteral injection of pollen extracts, what is called in clinical practice desensitization, results in the formation of antibody with the same specificity but with the general qualities of a classical circulating antibody, i.e., not readily fixed in the tissues, passing the placenta and relatively

heat stable (Loveless 1940). Presumably by preferential union with the antigen, sensitization phenomena are reduced or annulled in the presence of the second antibody. It is obvious therefore that the sensitizing antibody must arise by some mechanism distinct from that involved in the production of the classical "protective" antibody. There is no reason to doubt that sensitization results from a process initiated by the lodging of pollen grains on the respiratory mucosa. Two possibilities then present themselves to account for the characteristic type of antibody produced. (1) The fabrication of the antibody may be a function not of the relatively specialized mechanism in lymph nodes or spleen but of the collections of mesenchymal cells lying beneath the mucosa which usually include histiocytes, lymphocytes and variable numbers of plasma cells. (2) The special characteristics of the antibody may depend on the existence of a complex antigen formed by the union of a body protein with some component liberated from the pollen granule, a process which on this hypothesis does not occur when pollen extracts are introduced parenterally. The two alternatives are not incompatible since the second process might depend on the pollen granules being taken up by local histiocytes. The evidence for the existence of effective antigens which are complexes of the sensitizing agent and a body protein is derived almost wholly from studies of eczematous sensitization and will be considered later.

Eczematous sensitization is of particular interest because in most cases at least the sensitizing substance is of simple chemical character and hence incapable as such of acting as an antigen. It has been generally considered for some time that the effective antigen is a conjugate of the sensitizing agent with a body protein. This concept has been placed on a sound basis by the work of Gell, Harington and Rivers (1946), who showed that a number of typical sensitizing agents would combine with protein in ways analogous to those in which "synthetic" antigens had been prepared by Landsteiner and his followers.

No circulating antibody has been demonstrated in eczematous sensitization although the generalization of

reactivity to the whole of the skin demands that in some form or other the antibody does circulate. Chase (1946) has demonstrated successful passive transfer of sensitization of this type by cellular exudates and tissue from the spleen and lymph nodes of sensitized animals. It is evident therefore that the antibody is very readily taken up by cells and possibly circulates on some cellular carrier. To account for this character, Rostenberg (1947) has suggested that in the typical dermal sensitization the protein with which the sensitizing agent combines to form the effective antigen is one typical of dermal tissue. The antibody formed has a double specificity, one part of which is directed toward a body cell component, the other toward the sensitizing hapten. Circulating molecules of antibody will therefore be selectively adsorbed to cells on whose surface the body component is present. It is conceivable but far from certain that the capillary endothelium of a given organ such as the skin may have some organ-specific qualities allowing for the selective adsorption of such an antibody type as Rostenberg postulates. On such a hypothesis the sensitizing antibody might be produced by the same macrophage-lymphocyte system as classical antibody, the difference depending not on the mode of production but on the existence of a self- as well as a foreign-component in the presenting antigen.

The weight of evidence seems rather against this conception, particularly in view of the implications of Raffel and Forney's (1948) work on the influence of tubercle bacillus wax on the sensitizing capacity of picryl chloride already referred to. It seems more likely that reactions of the sensitizing chemical with protein and lipid components of the tissues into which it is introduced will produce immunologically active complexes of more than one sort. The particular type of sensitization and antibody produced will then depend on the physical character of the complex insofar as this determines what types of cell will be involved in initiating the immunological process. Rostenberg also mentions this possibility, considering that, if the conjugate is insoluble and is taken up locally in a region such as the skin where macrophages are abundant,

it will result in sensitization of eczematous type. If circumstances are such that a soluble conjugate is formed it may reach the general antibody-producing mechanism and give the immediate type of sensitization.

Further evidence on this point may be obtained from Chase's (1946) finding that when a sensitizing chemical, 2:4 dinitrochlorobenzene, was previously fed to guinea pigs this specifically prevented or greatly reduced the development of cutaneous sensitization by the usual procedures. This suggests that passage through the gut wall gives rise to a conjugate capable of provoking antibody equivalent in some ways to the "protective" antibody produced by parenteral injection of pollen extracts. Chase (1949) however finds that guinea pigs treated in this way can be passively sensitized by cell transfer and considers that their refractoriness to sensitization cannot be due to the presence of a blocking antibody. The possibility that the phenomenon is analogous to the blocking of subsequent response by the administration of a large initial dose of pneumococcal polysaccharide may have to be considered.

The tuberculin reaction has been studied more extensively than any other type of immunological sensitization and shows many points of similarity to the eczematous reaction. From the general immunological point of view there are four important features of the reaction:

(1) Any portion of the skin of a tuberculous guinea pig will show the characteristic reaction to tuberculin injection, and there is evidence from tissue culture studies that cells from lymph nodes or spleen are also specifically sensitive. This general sensitization of most or all tissues indicates that the sensitizing agent is distributed by the blood.

(2) Tuberculin sensitivity does not appear immediately after infection, but at a time (seven to fourteen days), which is comparable to that at which many types of antibodies first become demonstrable.

(3) The reaction is specific, being shown only by tuberculous animals. There is even some evidence that there are specific differences in the sensitization produced by bovine and human infections.

(4) While tuberculin sensitivity can only occasionally be passively transferred with serum, e.g. Zinsser and Mueller (1925), Chase (1945) achieved passive transfer with cells from the peritoneal exudate, the spleen, or the lymph nodes of a tuberculin-sensitive guinea pig. Tuberculin sensitivity was established in the normal animal three days after the intraperitoneal inoculation of the cell suspension, but persisted for only a few days.

The most satisfactory interpretation of these facts is that the sensitizing antigen is taken up by cells and sets in train the production of antibody molecules differing from normal antibody in being taken up by the general vascular endothelium and by any other cells with which they may make contact. Passive transfer of tuberculin sensitivity by cell suspensions may be due to the elaboration of antibody by the transferred cells during their residence in the recipient.

The outstanding feature of tuberculin sensitization is in fact the *cellular* locus of almost all the phenomena concerned. It is the only immunological condition in which it is possible to demonstrate with regularity that cells in tissue culture from a sensitized animal are killed or damaged by contact with the antigen. Moen and Swift (1936) showed that the sensitivity of splenic cells from a tuberculous guinea pig persisted in tissue culture for three generations. Moen (1936) demonstrated a similar capacity with tissue cultures derived from a small number of pleural exudate cells from a tuberculous animal. Quantitative considerations excluded the persistence of non-living antigen through many generations of cell multiplication and careful cultural and guinea pig inoculation experiments proved that there were no viable tubercle bacilli in the tissue cultures. As far as the evidence is available no other type of sensitization shows this behaviour. Washed cells from rabbits showing Arthus type sensitization are unaffected by the corresponding antigen and the same holds for human cells from hay fever patients (Rich 1941).

The site of production of the sensitizing antibody has not been demonstrated with certainty but the available evidence points toward the cells immediately associated

with the tuberculous lesion. Dienes and Schoenheit (1927) have shown that when egg albumin is injected into a tuberculous lesion in a guinea pig a tuberculin type of hypersensitiveness to egg albumin develops, quite different in character from the reactivity induced by the same antigen in a normal guinea pig. Unlike the true anaphylactic response, this cannot be transferred passively to normal animals. To obtain a regular sensitization of this type it is necessary to inject the antigen into the local tuberculous lesion, and Dienes (1929) finds that the best results are obtained if the lesion is first injected with a dose of tubercle bacilli, resulting in a rapid enlargement of the focus, and then two days later the egg albumin is injected into the inflammatory mass. The induced hypersensitiveness to egg albumin therefore seems to be definitely related to the placing of the antigen in an inflammatory area in which histiocytes and lymphocytes predominate.

Raffel and Forney (1948) refer to unpublished experiments in which they have shown that purified wax of the tubercle bacillus when administered with egg albumin will induce delayed-type sensitization. They indicate that "the lipid and the antigen must be in rather intimate contact within the body for the alteration (to the delayed type of response) to occur". They are much more impressed with the likelihood that interaction between the antigen and the wax gives rise to a complex of new antigenic potentiality, i.e., capacity to give delayed-type sensitization to any antigen, rather than with the alternative that the presence of the wax calls forth a local accumulation of cells which will initiate the production of the sensitizing antibody. We see no reason why both factors should not co-operate in producing the result.

Burky (1934) has described a somewhat similar effect in which sensitization of rabbits to certain antigens which alone do not produce sensitization was accomplished by inoculating the antigen with staphylococcal toxin. Amongst the antigens used were ragweed pollen and lens protein. Lucic (1939) has obtained sensitization of rabbits to uveal tissue by the same method. It is natural to consider that antibody produced actually by the inflammatory cells

in the lesion is responsible for the sensitization, both against tuberculin and against any second antigen inoculated into the lesion.

Some support for this view may be drawn from the fact that while tuberculous rhesus monkeys do not normally become tuberculin reactive, Casals and Freund (1939) found that injections of killed tubercle bacilli in paraffin oil which produced large local lesions gave rise to well marked tuberculin hypersensitiveness. If, as recent work seems to suggest, sarcoidosis in human beings is a type of tuberculous infection unassociated with tuberculin reactivity, comparative histological study of the lesions in the two diseases may throw light on the cellular components responsible for the production of sensitization.

This conclusion is strengthened by a consideration of other types of bacterial sensitization which have been studied in sufficient detail. The hypothesis that acute nephritis and many rheumatic conditions may represent allergic reactions to the products of streptococcal infection has led to an extensive experimental study of the development of skin reactivity in rabbits infected by various types of streptococci. Derick and Swift (1929) conclude that hypersensitiveness to *viridans* streptococci in rabbits is seen only when a local focus of infection exists; it does not follow intravenous injection. Further, there is no close correlation between hypersensitiveness and the presence in the blood of antibody of any of the types ordinarily tested. After intravenous injection of the same streptococci a state of relative immunity to skin infection develops. The lesions are smaller and more compact, and do not give rise to the hypersensitive state as they do in normal rabbits. The experiments involve the use of very complex antigenic material (living streptococci), but there is a distinct indication that two types of antibody, sensitizing and immunizing, are concerned. Angevine's (1939) experiments point in the same direction. Virulent and relatively avirulent sub-strains were developed from a single strain of haemolytic streptococci. Injected into the rabbit skin, the former produced severe lesions with septicaemia and infection of internal organs such as the

spleen. Recovery was followed by immunity. The avirulent strain produced a more indolent lesion, and gave rise to the same type of skin hypersensitiveness as was described by Derick and Swift. In both series of experiments it seems reasonable to assume that the sensitizing antibody (or agent) was produced in the local lesion, the immunizing antibody by the general antibody-producing mechanism, i.e., the reticulo-endothelial cells of spleen, liver, bone marrow and lymph nodes.

Julianelle's (1930) studies on the response of rabbits to the intradermal injection of killed pneumococci produced generally similar results. Such injections fail to produce type-specific antibodies, but give rise to (a) an increased resistance to infection with any type of pneumococcus, (b) an increased local reaction to subsequent injections of killed cocci, (c) a reaction resembling the Arthus phenomenon following intradermal injection of a "nucleoprotein" fraction, and (d) a slow appearance of antibody giving a precipitin reaction with nucleoprotein. Except for the certainty that the type-specific polysaccharide is not involved, it is not clear which, or how many of the other antigens are concerned in the different reactions. Dubos and MacLeod's (1938) work on the effect of leucocytic extracts in destroying the antigenicity of the type-specific antigen provides a partial explanation of the failure of type-specific antibodies to appear after intradermal inoculation. It is probable that the cellular response to such inoculations of dead pneumococci would include both polymorphonuclears and histiocytes, and it would be of interest to know whether the latter cells had the same destructive effect on the type-specific antigen as the polymorphonuclears.

A number of other "skin tests" depending on acquired hypersensitiveness to some product of an infecting micro-organism have been described. These are particularly characteristic of infections producing sub-acute or chronic lesions in which histiocytes play an important part. Brucellosis is a typical bacterial example. Amongst the virus infections lymphogranuloma inguinale is the only one giving relatively intense skin sensitization to the appropriate an-

tigen (Frei test). Mumps is also associated with a well defined but less intense skin sensitivity (Enders, Cohen and Kane 1945), while herpes simplex and influenza in which the local lesions are associated with relatively little monocytic cellular reaction, give recognizable but still weaker reactions (Beveridge and Burnet 1944, Nagler 1944). Sensitization may sometimes be induced by injection of large doses of dead bacteria, but probably only when a definite local lesion is produced. Petroff and Stewart (1925), for instance, found that guinea pigs could be rendered sensitive to tuberculin by inoculation of large doses of killed tubercle bacilli by any route except the intravenous one.

In discussing the interpretation of the phenomena of sensitization in relation to the general concept of antibody production developed in the preceding chapter, we are limited by the virtual impossibility of carrying out detailed experiments of the type available for the study of easily measured circulating antibodies. Opinions as to the types of cell which are involved in producing the sensitizing agents must therefore be based on indirect evidence and are largely speculative.

Our basic hypothesis is that sensitization is always a result of the production and liberation of antibodies which are either identical with classical circulating antibody as in guinea pig anaphylaxis or differ only in the greater ease with which they are taken up by cells. This difference is only of moderate degree in the case of hay fever type sensitization but in delayed-type sensitization the difference is so great that only undetectable amounts are ever present in the circulating blood.

Sensitization of the delayed type is quite regularly associated with sub-acute accumulations of inflammatory cells in the site of application of the sensitizing agent. These accumulations include histiocytic cells (macrophages, endothelioid cells), primitive mesenchymal cells, lymphocytes and plasma cells and all the evidence is compatible with the view that the sensitizing antibody is produced within this inflammatory cell complex. We believe that, depending in part on preliminary modification of antigen

including formation of antigen by combination of simple substances with protein and perhaps union with lipids such as those of the tubercle bacillus, and in part on the nature of the cells available, a characteristic type of antibody is produced. The process is assumed to be essentially the same as that taking place in the specialized antibody-producing organs of lymph nodes and spleen, i.e., a joint activity of macrophages and undifferentiated mesenchymal cells which in the process take on plasma cell character. The modified units produced are liberated as complete rather than partial replicas of the intracellular units and judging from the tissue culture experiments of Moen they retain the capacity for replication when transferred to a variety of cells. This characteristic may well be a primitive one which has been refined and specialized for the production of circulating antibody in the organs developed for that function.

The characteristics of hay fever type antibody are clearly intermediate between those of classical and "delayed-type" antibodies. The antibody is readily attached to tissues but it cannot sensitize cells in tissue culture and is regularly detectable in the circulating plasma. It seems likely that sub-epithelial mesenchymal cells in the mucous membranes exposed to the sensitizing agent are in some way concerned. Perhaps the initial development of the modified intracellular unit takes place in local macrophages and this unit is subsequently transferred to the reticulum cells of the draining lymph nodes. Other possibilities might be thought of but it is sufficient to conclude that its mode of production as well as its characteristics, will eventually be found to be intermediate between classical and delayed-type antibodies.

CHAPTER X

TISSUE TRANSPLANTATION IMMUNITY IN RELATION TO IMMUNOLOGICAL THEORY

If our contention is correct that the antibody-producing mechanism has a dual function which also includes the disposal of worn out or damaged body cells, the conditions which arise when foreign cells and tissues from the same or related species are introduced into the body become of particular interest. The complications arising in the course of blood transfusion have given a special stimulus to the study of blood cell differences. In part these reactions are explicable on normal immunological principles, i.e., circulating antibodies (agglutinins and haemolysins) are produced in response to an antigenic pattern foreign to the recipient but in addition and of much greater practical importance there is the existence of the normal isoagglutinins to be considered. This represents one of the major problems to be covered by any immunological theory. The isoagglutinins have a general resemblance to immune haemagglutinins but there is no indication that they are produced immunologically in any ordinary sense. As a rule they do not appear until some days after birth and gradually increase in titre until adolescence after which the isoagglutinin titre tends to fall. Those agglutinins are present which have no action on the subject's own red cells. Group A subjects have β isoagglutinin, Group O α and β and so on. Current interpretation of these facts is virtually reduced to a statement that the genetic constitution of the individual determines both his blood group and the isoagglutinins in his serum. It seemed possible that the self-marker concept developed in the preceding chapter could throw some light on this problem.

In an attempt to develop a satisfactory hypothesis along these lines we have been forced to consider as well the origin of other "natural antibodies" e.g. those causing

agglutination or haemolysis of some foreign red cells and of γ globulin which has no specific immunological character. In the absence of direct evidence it is necessary to assume, as all writers on the subject have done, that these are produced by essentially the same mechanism as antibodies. In terms of our own hypothesis they are products of unmodified "primary units". The pseudo-immunological patterns carried by normal antibodies, including the isoagglutinins, can only be ascribed to the "accidental" presence of a configuration complementary to the antigen concerned. This is probably acceptable to all as far as the heterologous agglutinins for foreign red cells are concerned but doubts might be raised in regard to the isoagglutinins. The chief justification for accepting the "accidental" point of view is that isoagglutinins of human type are rare in other mammalian species and are apparently unknown in birds. It is therefore very difficult to see what positive function the isoagglutinins can serve in the human species or how they could have been developed by normal evolutionary processes. Future work may provide a more acceptable interpretation than simple accident but no such interpretation is now visible. Although the potential capacity to produce α and β isoagglutinins may be essentially accidental it is obvious that there is nothing accidental in the inhibition of the liberation of those which are incompatible with the circulating cells.

To interpret this inhibition in terms of the self-marker hypothesis requires a slight extension of the conception of how the "recognition unit" is developed during embryonic life. We assume that in the primitive mesenchymal cells which carry out scavenging functions in embryonic life there are "primary units" which will eventually give rise to those units which in the free-living animal produce the various types of γ globulin including the normal antibodies. If an expendable cell carries an antigen which corresponds (by the accident of a complementary configuration) with one of these units the latter will be converted into a "recognition unit" which by definition cannot be liberated into the circulation. By the time adult life is reached it must be presumed that all the scavenging cells

of the body have by one means or another acquired this unit. Where no corresponding antigen exists in the body cells the primary unit undergoes unmodified development and at the appropriate stage of bodily maturity contributes its characteristic globulin to the circulating pool.

In a human individual of blood group A the embryonic cells contain potential producers of α and β isoagglutinins. The A antigen however, present from the second month of embryonic life (Kemp 1930), converts the potential α isoagglutinin producer into a "recognition unit". The β producer is unchanged and a week or two after birth begins to liberate the β agglutinin. Similar reasoning will cover the conditions found in AB, B and O group individuals.

The conditions governing the development of recognition units can now be stated more generally in the following fashion: The cell component which is the potential marker may find either a pre-existent unit (globulin producer) which has a complementary structure already formed (either by accident or because of some as yet unrecognized relationship) or a unit which requires appropriate modification before the complementary pattern is developed. In either event the units are stabilized and after birth take no part in the liberation of plasma globulin. Such a general conception would fit in well with a supposition that the lymphocyte or plasma cell is essentially a nurse cell and transporter of globulin whose pattern had been determined within the reticulo-endothelial cell. The characteristic that defines the self-recognizing units is that they are *not* transferable to the transporting and liberating cells.

The second type of tissue transplantation that requires further discussion in relation to general antibody theory is transplantation of somatic tissues as typified by skin grafting. The extensive studies of Medawar (1944-45) on skin grafts in rabbits provide conclusive evidence that the failure of heterologous grafting is due to immunological factors. The important points arising from his work are (1) In a normal recipient, tissue from another animal attaches and grows normally for about ten days before it separates and necroses. (2) An animal that has reacted in

this way responds much more rapidly against a second or subsequent graft from the same donor but not from a different donor. (3) The intensity and speed of reaction against a second graft is directly related to the size ("dosage") of the initial graft. (4) The immune state produced by the primary graft is generalized over the whole skin surface and is not confined to the neighbourhood of the primary site.

The obvious interpretation of these facts is that in response to the primary stimulus an immunologically altered state of reactivity is induced which in 12-14 days results in destruction of the graft. A second graft from the same source finds the reactive state already established and the destructive process begins as soon as the graft cells commence to multiply.

The nature of the immunological response is still obscure. Medawar (personal communication) was unable to demonstrate the existence of serum antibodies using passive transfer and tissue culture techniques. No damaging effects could be shown on tissue cultures of donor rabbit skin from the action of sera, cells or tissue extracts of rabbits immunized by the repeated application of large grafts. Rabbits could be immunized against skin grafting not only by a previous skin graft but also by intradermal (but not intravenous) injection of leucocytes from the donor animal. Histological studies of first- and second-set homografts indicated that the essential feature of the immune state was failure of cell division in the homografts.

A different approach to the problem is due to Harris (1943a and b) who carried out experiments with roller tube tissue cultures of various organs of rats and mice. He found that mixed cultures of rat and mouse cells survived with normal morphology in normal rat serum. If however serum from rats which had previously been immunized with mouse tissues was used in such mixed cultures, specific destruction of the mouse cells resulted. The significant point of these experiments may be the necessity of recipient-type cells to be present to allow an immunological effect on donor cells.

Two regions of the body have long been recognized as

especially favourable sites for successful tissue transplantation, namely the brain and the anterior chamber of the eye. Many investigators (e.g. Murphy 1926, Tansley 1946) have shown that foreign homologous tissue grafted to the brain of normal animals survives and similar success has attended many experiments on transplantation to the anterior chamber of the eye (Greene 1941a, Greene and Murphy 1945, Cheevers and Morgan 1942). Medawar (1948) carried out experiments upon the growth of skin grafts in the brain and anterior chamber of the eye of specifically immunized rabbits, using skin homografts from the same donor to a prepared site on the skin of the same recipient to control his observations. He found that under such conditions skin homografts to the brain were destroyed while those to the anterior chamber of the eye were destroyed if, and only if, they were vascularized. The anatomical peculiarities of these regions are the absence of any lymphatic drainage system in the brain and the fact that in the anterior chamber of the eye grafts may, as *in vitro*, be kept alive without penetration by blood vessels. Medawar concluded that a lymphatic drainage system is necessary to create a state of immunity but is not necessary to enforce a response to it. The immune response is mediated by the blood stream.

Several points arising from the phenomena of tissue graft immunity call for comment at the theoretical level. The first is the nature of the antibody concerned. A close relationship to sensitization processes is suggested by three facts: (1) the absence of detectable circulating antibodies; (2) the generalization throughout the skin surface of potentiality of immune response and (3) the necessity for intradermal as against intravenous immunization with leucocytes in rabbits. A working hypothesis is that the mechanism involved is similar to that postulated for tuberculin hypersensitivity. Antibody is produced in response to foreign antigenic compounds which are transferred to a wide variety of cells throughout the body. When a second graft from the same donor is implanted the effective antigen is liberated from donor cells, perhaps when they commence to multiply. When it comes into contact

with host cells there may well be a local liberation of the same pharmacologically active substances as are responsible for the necrosis of a fully developed tuberculin reaction. This suggestion appears susceptible to experimental study, e.g., by using extracts from donor cells (leucocytes, etc.) to test skin reactivity in rabbits immunized by skin grafts from the same donor.

The high specificity of the immune reaction which in manageable numbers of genetically unrelated rabbits is limited to a single donor (Medawar 1945) has some bearing on the marker hypothesis. The specific character of the skin graft is shared by leucocytes from the same animal. This suggests strongly that a small number only of specialized components common to both (or all) types of cell are concerned, A, B and C say, but that different individuals within the species have components which differ significantly from those of others. There might for instance be five "alleles" of each of A B C, so that two rabbits might have the composition A^2 B^1 C^5 and A^3 B^1 C^2. On such a basis a very large number of immunological types would be possible. The alternative that virtually all the complex molecules of the cells concerned have immunological specificity and that the net difference amongst all these components is reflected in the immunological response from their interaction seems most unlikely. The failure of immunization by implantation of foreign tissue within the central nervous system is related by Medawar (1948) to the absence of any lymphatic drainage system. It is known from Morgan's (1947a) work that antibody against poliomyelitis virus can be produced in the central nervous system and is extractable from the infected grey matter of the cord but it is characteristic of poliomyelitis in monkeys that antibody takes months to appear in the circulating blood. From Medawar's results one must deduce that not only is there no general liberation of antibody but also that any locally produced antibody is incapable of inhibitory or destructive action against the foreign tissue. There is a distinct suggestion that for the effective production of antibody the specialized regions exemplified by the lymph nodes and the spleen must play

some part but it is obvious that a great deal of further experimental work is called for.

TUMOUR IMMUNITY

The immunological basis of the destruction of homologous and heterologous tumour transplants is probably similar to that postulated for skin grafts but is complicated by the abnormal character of the transplanted cells. Such experiments as those of Furth, Boon and Kaliss (1944) which illustrate the great variability of the transplantation pattern of different tumours in pure lines of mice and their hybrids and the uniform behaviour of normal tissues, suggest that the cause for the variation may be in the different type of response by the host. It would be reasonable to assume that an essential requirement for transplantability of tumour to fresh hosts is the disappearance of the cell "markers" by a process of somatic mutation. On the other hand it is equally possible that the ability to continue growth in a foreign host is due to lack of susceptibility of the tumour cells to the agent or process that inhibits cell multiplication of a skin graft on an immunized animal.

CHAPTER XI

SUMMARY AND CONCLUSIONS

It will be obvious that this attempt at a comprehensive discussion of antibody production is hampered in all directions by lack of knowledge. Some of the wanted facts could be obtained by available methods; others will have to wait until new ideas and techniques arise. In this concluding chapter we have attempted to provide a summary of our theoretical approach. The main virtue of any such theory is that it sometimes allows a clearer view of the gaps in our knowledge of the subject than could be obtained without such a formulation and we have tried to indicate such gaps and weaknesses.

GENERAL ASPECTS OF THE THEORY OF ANTIBODY PRODUCTION

1. The basis of our account is the recognition that the same system of cells is concerned both in the disposal of effete body cells (without antibody response) and of foreign organic material (with antibody response).

2. In order to allow this differentiation of function expendable body cells carry "self-marker" components which allow "recognition" of their "self" character. Antigens in general are substances of the same chemical nature as the marker components but of different molecular configuration.

3. The initial stage of antibody production is regarded as the secondary adaptation of an intracellular enzymic unit, whose primary configuration is toward one or other "self-marker", to a configuration allowing specific adsorption to and initial enzymic destruction of the antigen.

4. The "enzymic units" so modified are capable of replication when stimulated by renewed contact with the antigen quite independently of genic control. They may be transferred to other cells, the range of accepting cells varying with the type of antigen and its mode of entry into the body.

5. Typical circulating antibody molecules are regarded as partial replicas of the modified enzymic units carrying their configurational specificity but lacking enzymic action and the capacity of transfer to unspecialized cells.

6. The production of intracellular units and their partial replicas (antibody) may continue for long periods after the antigen has disappeared from the body. Their activity and specificity steadily decline in the absence of a fresh stimulus by the same antigen but conversely with further such stimuli there is accelerated activity and the possibility of further change in the specificity of the antibody produced.

7. In the extreme case of tuberculin hypersensitivity the modified units retain their power of replication when transferred to unspecialized mesenchymal cells.

The weakness of such a formulation centres on the postulated intracellular enzymic unit and its capacity to undergo adaptive modification. This goes far beyond what is known of classical enzyme chemistry. There is no well studied instance of an enzyme with a specificity of action of a type comparable to antigen-antibody specificity but equally there are no present modes of studying such enzymes did they exist. There is a growing impression that self-replicating systems of the type postulated are an essential feature of cytoplasmic structure and that their synthesis is dependent on a complex associated mechanism in which ribonucleic acids play a vital part. There is no evidence from other than immunological fields that intracellular enzymes can be adapted to a wide range of substrates. The capacity of a bacterium or yeast to develop adaptive enzymes after appropriate contact with a substrate is strictly limited and in general is as definite a character of the strain as the range of its constitutive enzymes. Again, the argument is weakened by the present necessity of confining studies on adaptive enzymes to simple extracellular activities that can be readily measured by standard chemical procedures. As indicated in the text problems of the same level are presented by the relation

between gene and cytoplasmic constituents and by the process of cell differentiation.

The outstandingly controversial point in our hypothesis is the assumption that antibody production can continue in the absence of antigen within the body. Northrop has recently discussed this question and is fully alive to the importance of the point. In his view, based on unpublished experiments by Price, the manifest antigen, e.g. a virus or in Price's experiments hexokinase, is destroyed rapidly but gives rise to a breakdown product or products which are the effective and persisting antigen. There is only the most slender evidence for even the temporary existence of such secondary antigens in the spleen and none for their prolonged retention. We would reiterate our contention that in the dynamic constantly changing cellular population of spleen or lymph node there is no storage place for either intact antigen or any hypothetical "template" derivative. However, it still seems possible that direct experimental decision might be obtained with a long term radioactive isotope, e.g. C_{14} incorporated in a known determinant group of the antigen and this should be sought.

At a less fundamental level is the view of Ehrich (1946) that the function of the macrophage is to break down the complex antigen to effective antigenic fragments which are then passed on to the actual antibody producer either, in his view, the lymphocyte or, in ours, the reticulum cell which while producing antibody takes on the plasma cell character. This process if it occurs might be experimentally detectable but the evidence of Harris and Ehrich (1946) discussed in Chapter V is not valid since the soluble antigenic material passing from the local depot to the lymph node might still be taken up by the lymph node macrophages.

A serious weakness in our general presentation is the failure to account for the importance of the macro-molecular "carrier" of the antigenic determinants of specificity. This is normally a protein or the whole complex of organic structure in the case of particulate micro-organismal or cellular antigens. In the general discussion this character

of complete antigens has been tacitly accepted as a necessary feature of antigenicity. The simplest interpretation is that the macro-molecular character is needed to allow the antigen to be taken up by the appropriate antibody-producing cells. There may be a secondary necessity for a large relatively non-diffusible unit to allow effective contact with a complex intracellular unit. The function of the carrier seems to be of special importance in the phenomena of sensitization by simple chemical substances which can react readily with protein. Since the effective antigen evidently includes protein of the subject's own tissues, studies in this field might lead to important decisions as to the utility or otherwise of the self-marker hypothesis and as to the part played by the carrier in determining the type of antibody produced.

The self-marker concept seems to provide a number of suggestions for experimental work to substantiate or refute it. A virtually direct proof of its correctness could be obtained if experimental techniques could be developed to produce with a wider range of antigens introduced into embryos the persisting tolerance of foreign cells found by Owen in his studies on multiple births in cattle. The possibility of finding substances which by combining with or otherwise modifying the markers on circulating red cells could render them antigenic has been suggested in regard to blackwater fever (Gear 1946) and might equally be considered for some other acute haemolytic anaemias. It has been shown in this laboratory that a new antigen is produced on red cells by the action of influenza viruses or a vibrio enzyme (Burnet and Anderson 1947) and this may indicate a possible general approach to the wider question of self-markers on expendable cells. The use of skin grafting experiments for the analysis of individual specificity, including the chemical identification of the antigenic components, is another field in which the technical difficulties should not prove insuperable.

CELLULAR ASPECTS OF THE THEORY

1. The antibody-producing mechanism is initiated by the entry of the antigen into phagocytic cells of the

reticulo-endothelial system (macrophages). In these cells the conversion of the intracellular units to adaptively modified units takes place.

2. These units are transferred to reticulum cells or other relatively undifferentiated mesenchymal cells in the immediate vicinity of the macrophages.

3. Under conditions inducing active antibody production these cells or some of them become active in protein synthesis and take on the staining qualities characteristic of plasma cells. They multiply freely and are responsible for the actual production of circulating antibody during the peak phase of the immunological response.

4. The same reticulum cells give rise to lymphocytes which also carry antibody-producing units in much less active state. The lymphocytes are probably responsible for maintenance of low levels of antibody long after the antigenic stimulus.

5. Sensitization phenomena are characteristically associated with macrophage accumulations at the site of injection plus the associated undifferentiated and differentiated mesenchymal cells. A less specialized product is produced which is more readily attached to tissue cells and in the limit is capable of replication within them.

There is no real evidence that the antibody-producing units are built up in the macrophages. Since by hypothesis these units do not produce antibody until they are transferred to suitable "nurse cells", direct evidence on the point is unobtainable. Our location of the primary mechanism here is based wholly on circumstantial evidence. If methods could be developed for the histo-chemical study of antigen changes taking place within the cell, a decision might be possible between our view and the alternative that in the macrophage the crude antigen is broken down to the effective antigen which is passed on to another type of cell which produces the antibody.

Our whole-hearted acceptance of the Scandinavian view that the plasma cell is the predominant antibody producer may be proved unwise by future work. The evidence is still largely circumstantial but our own current studies of

the plasma cell response in the spleen of fowls being immunized are so completely in line with those of Fagraeus in the rabbit that we find it hard to believe that these cells are not the actual producers and liberators of antibody. Logically there is still the possibility that the protein synthesizing activity manifested by the staining method may be merely a preliminary stage in the synthesis of globulin which is then transferred, perhaps to lymphocytes where it receives the antibody stamp.

The function of the lymphocyte is still obscure and our picture of it as a "second string" to the plasma cell as transporter and liberator of antibody is not drawn with any conviction. If it is a correct picture the immunological function of the lymphocyte can hardly be its major one. The consistent failure to show any immunological activity in thymus lymphocytes (Harris, Rhoads and Stokes 1948) also points in this direction.

It must be stressed that our discussion of cellular aspects of antibody production is based on experiments with a limited range of particulate antigens capable of giving an easily measurable antibody response. There is sufficient evidence to show that antitoxin production differs very sharply from bacterial agglutinin production in the rabbit. In the fowl, which responds well to the usual particulate antigens, there is almost no response to the inoculation of diphtheria or staphylococcal toxoid. It is evident that in the case of this important type of antibody production, the interpretation summarized above is inapplicable. Studies to elucidate the cells concerned in antitoxin formation seem urgently called for.

In the first edition of this monograph we pointed out that our experiments with staphylococcal toxoid indicated that this antigen had two separable functions: (1) To impress a modified character on the antibody-producing cells; (2) to stimulate the output of modified globulin (antitoxin) from the cells which have undergone this primary modification. The suggestion was made that renewed contact with the antigen must serve as a stimulus to general cell activity, including proliferative activity. This opens up a further field which will need to be

explored if any quantitative relation between cell processes and antibody production is to be established. It may be that secondary contact releases histamine or other pharmacologically active substances in the modified cell and that these agents are directly responsible for the cellular activity and proliferation.

Two types of cell have been completely neglected in our discussion of antibody production, the polymorphonuclear leucocyte and the eosinophil. Both will probably need to be considered in any adequate account but at present there seems to be no way of fitting them into the picture. The eosinophil is undoubtedly associated in some way with sensitization processes and the elucidation of the connection will help greatly in the understanding of sensitization. Is it possibly a carrier of one type of sensitizing antibody? The question might be answered by seeing whether washed leucocytes from a human allergic patient with very high eosinophilia could be used to transfer sensitivity to a normal individual by the Prausnitz-Kustner technique.

ANTIBODY PRODUCTION IN THE LIGHT OF GENERAL BIOLOGICAL THEORY

We have used the analogy of the adaptive enzyme as the best means of visualizing the antibody-producing mechanism but we should stress that the analogy with adaptive enzymes splitting simple sugars is by no means close. In yeasts and bacteria the range of sugars to which adaptive enzymes can be developed is strictly determined by genetic factors. There appears on the other hand to be virtually no limit to the number of minor variations in pattern of effective antigens within the broad limitations which determine antigenicity as such. Further, the influence of genetic factors on antibody-producing ability seems to be very slight. The adaptive enzyme analogy is the best we can provide but it is perhaps legitimate to suggest that antibody formation is a more fundamental biological phenomenon than adaptive enzyme production by unicellular organisms. If the type of specific complementary pattern adsorption *known* to occur between antigen and

antibody *in vitro* can legitimately be postulated, as we have done for the self-replicating antibody-producing unit within the cell, the concept may be of value for the interpretation of a variety of intracellular phenomena. The idea that before certain enzymic actions take place specific complex pattern adsorption is required may well be helpful in understanding intracellular organization. Work with which one of us has been concerned on the nature of the enzymic action of influenza virus on certain mucopolysaccharides seems to require such a concept to interpret that reaction. Until the various levels of biological thought can be brought together in terms of a unified system of concepts each sub-science has the right to introduce its own generalizing concepts. Immunology has through most of its history been remote from the general stream of biological discovery and generalization. This attempt to bring it more directly into that stream is equally an attempt to show that immunological phenomena and interpretations must be given due weight in any future formulations of the nature of living process.

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